

PCT

WORLD INTELLECTUAL
PROPERTY ORGANIZATION
Intern:

INTERNATIONAL APPLICATION PUBLISHED IN



WO 9602273A1

(51) International Patent Classification 6:

A61K 39/42, C07K 16/10, G01N 33/577

A1

(43) International Publication Date: 1 February 1996 (01.02.96)

(21) International Application Number: PCT/US95/08743

(22) International Filing Date: 11 July 1995 (11.07.95)

(30) Priority Data:
08/276,852 18 July 1994 (18.07.94) US

(71) Applicant: THE SCRIPPS RESEARCH INSTITUTE [US/US];
10666 North Torrey Pines Road, La Jolla, CA 92037 (US).

(72) Inventors: BURTON, Dennis, R.; 6044 Beaumont Avenue, La
Jolla, CA 92037 (US). BARBAS, Carlos, F.; 7081 Weller
Street, San Diego, CA 92122 (US). LERNER, Richard, A.;
7750 East Roseland Drive, La Jolla, CA 92037 (US).

(74) Agents: FITTING, Thomas et al.; The Scripps Research
Institute, 10666 North Torrey Pines Road, TPC-8, La Jolla,
CA 92037 (US).

(81) Designated States: AU, CA, FI, JP, NO, European patent (AT,
BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL,
PT, SE).

Published
With international search report.

(54) Title: HUMAN NEUTRALIZING MONOCLONAL ANTIBODIES TO HUMAN IMMUNODEFICIENCY VIRUS

(57) Abstract

The present invention describes human monoclonal antibodies which immunoreact with and neutralize human immunodeficiency virus (HIV). Also disclosed are immunotherapeutic and diagnostic methods of using the monoclonal antibodies, as well as cell line for producing the monoclonal antibodies.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

HUMAN NEUTRALIZING MONOCLONAL ANTIBODIES
TO HUMAN IMMUNODEFICIENCY VIRUS

Technical Field

5 The present invention relates generally to the field of immunology and specifically to human monoclonal antibodies which bind and neutralize human immunodeficiency virus (HIV).

Background

10 1. HIV Immunotherapy

HIV is the focus of intense studies as it is the causative agent for acquired immunodeficiency syndrome (AIDS). Immunotherapeutic methods are one of several approaches to prevention, cure or remediation of HIV infection and HIV-induced diseases. Specifically, the use of neutralizing antibodies in passive immunotherapies is of central importance to the present invention.

Passive immunization of HIV-1 infected humans using human sera containing polyclonal antibodies immunoreactive with HIV has been reported. See for example, Jackson et al., Lancet, September 17:647-652, (1988); Karpas et al., Proc. Natl. Acad. Sci. USA, 87:7613-7616 (1990).

Numerous groups have reported the preparation of human monoclonal antibodies that neutralize HIV isolates in vitro. The described antibodies typically have immunospecificities for epitopes on the HIV glycoprotein gp120 or the related external surface envelope glycoprotein gp120 or the transmembrane glycoprotein gp41. See, for example Levy, Micro. Rev., 57:183-289 (1993); Karwowska et al., Aids Research and Human Retroviruses, 8:1099-1106 (1992); Takeda et al., J. Clin. Invest., 89:1952-1957 (1992); Tilley et al., Aids Research and Human Retroviruses, 8:461-467 (1992); Laman et al., J. Virol., 66:1823-1831 (1992); Thali et al., J. Virol., 65:6188-6193 (1991); Ho et al., Proc. Natl. Acad. Sci. USA, 88:8949-8952 (1991); D'Souza et al., AIDS, 5:1061-1070 (1991); Tilley et al., Res. Virol., 142:247-259

(1991); Broliden et al., Immunol., 73:371-376 (1991);
Matour et al., J. Immunol., 146:4325-4332 (1991); and
Gorny et al., Proc. Natl. Acad. Sci., USA, 88:3238-3242
(1991).

5 To date, none of the reported human monoclonal
antibodies have been shown to be effective in passive
immunization therapies. Further, as monoclonal
antibodies, they all each react with an individual epitope
on the HIV envelope glycoprotein, gp120 or gp160. The
10 epitope against which an effective neutralizing antibody
immunoreacts has not been identified.

There continues to be a need to develop human
monoclonal antibody preparations with significant HIV
neutralization activity. In addition, there is a need for
15 monoclonal antibodies immunoreactive with additional and
diverse neutralizing epitopes on HIV gp120 and gp41 in
view of recent studies suggesting that gp120 and gp41 are
involved in both binding of the HIV virus to the cell as
well as in post binding events including envelope shedding
and cleavage. See, for review, Levy, Micro. Rev., 57:183-
20 289 (1993). Additional (new) epitope specificities are
required because, upon passive immunization, the
administered patient can produce an immune response
against the administered antibody, thereby inactivating
25 the particular therapeutic antibody.

2. Human Monoclonal Antibodies Produced From Combinatorial Phagemid Libraries

The use of filamentous phage display vectors, referred
30 to as phagemids, has been repeatedly shown to allow the
efficient preparation of large libraries of monoclonal
antibodies having diverse and novel immunospecificities.
The technology uses a filamentous phage coat protein
membrane anchor domain as a means for linking gene-product
35 and gene during the assembly stage of filamentous phage
replication, and has been used for the cloning and
expression of antibodies from combinatorial libraries.
Kang et al., Proc. Natl. Acad. Sci., USA, 88:4363-4366

(1991). Combinatorial libraries of antibodies have been produced using both the cpVIII membrane anchor (Kang et al., supra) and the cpiii membrane anchor. Barbas et al., Proc. Natl. Acad. Sci., USA, 88:7978-7982 (1991).

5 The diversity of a filamentous phage-based combinatorial antibody library can be increased by shuffling of the heavy and light chain genes (Kang et al., Proc. Natl. Acad. Sci., USA, 88:11120-11123 (1991)), by
10 altering the CDR3 regions of the cloned heavy chain genes of the library (Barbas et al., Proc. Natl. Acad. Sci., USA, 89:4457-4461 (1992)), and by introducing random mutations into the library by error-prone polymerase chain reactions (PCR) [Gram et al., Proc. Natl. Acad. Sci., USA, 89:3576-3580 (1992)].

15 Filamentous phage display vectors have also been utilized to produce human monoclonal antibodies immunoreactive with hepatitis B virus (HBV) or HIV antigens. See, for example Zebedee et al., Proc. Natl. Acad. Sci., USA, 89:3175-3179 (1992); and Burton et al.,
20 Proc. Natl. Acad. Sci., USA, 88:10134-10137 (1991), respectively. None of the previously described human monoclonal antibodies produced by phagemid vectors that are immunoreactive with HIV have been shown to neutralize HIV.

25 In particular, none of the previously-described human monoclonal antibodies produced by phagemid vectors are capable of neutralizing a majority of the field isolates of HIV. It is believed that certain of the antibodies described herein are particularly effective at
30 neutralizing HIV because the antibodies immunoreact with an important antigenic determinant present on "mature" gp120 and not present on the HIV precursor protein gp160.

Brief Description of the Invention

35 Methods have now been discovered using the phagemid vectors to identify and isolate from combinatorial libraries human monoclonal antibodies that neutralize HIV, and allow the rapid preparation of large numbers of

neutralizing antibodies of completely human derivation. The identified neutralizing antibodies define new epitopes on the HIV gp120 and gp41 glycoproteins, thereby increasing the availability of new immunotherapeutic human monoclonal antibodies.

The invention provides human monoclonal antibodies that neutralize HIV, and also provides cell lines used to produce these monoclonal antibodies.

Also provided are amino acid sequences which confer neutralization function to the antigen binding domain of a monoclonal antibody, and which can be used immunogenically to identify other antibodies that specifically bind and neutralize HIV. The monoclonal antibodies of the invention find particular utility as reagents for the diagnosis and immunotherapy of HIV-induced disease.

A major advantage of the monoclonal antibodies of the invention derives from the fact that they are encoded by a human polynucleotide sequence. Thus, in vivo use of the monoclonal antibodies of the invention for diagnosis and immunotherapy of HIV-induced disease greatly reduces the problems of significant host immune response to the passively administered antibodies which is a problem commonly encountered when monoclonal antibodies of xenogeneic or chimeric derivation are utilized.

An additional major advantage of a preferred group of monoclonal antibodies described herein derives from the fact that they immunoreact with a unique determinant present on mature HIV glycoprotein gp120. This class of antibodies is particularly effective at neutralizing field isolates of HIV.

In one embodiment, the invention contemplates a human monoclonal antibody capable of immunoreacting with human immunodeficiency virus (HIV) glycoprotein gp120 and neutralizing HIV. A preferred human monoclonal antibody has the binding specificity of a monoclonal antibody comprising a heavy chain immunoglobulin variable region amino acid residue sequence selected from the group consisting of SEQ ID Nos 66, 67, 68, 70, 72, 73, 74, 75,

78 and 97.

In a particularly preferred embodiment, the invention describes a human monoclonal antibody capable of immunoreacting with human immunodeficiency virus (HIV) glycoprotein gp120 and neutralizing HIV, wherein the monoclonal antibody has the capacity to reduce HIV infectivity titer in an in vitro virus infectivity assay by 50% at a concentration of less than 700 nanograms (ng) of antibody per milliliter (ml).

Preferably, an anti-gp120 monoclonal antibody of this invention binds mature gp120 preferentially over HIV precursor glycoprotein gp160. More preferably, an anti-gp120 monoclonal antibody binds to a V1/V2 loop deficient-variant gp120 substantially less than native gp120, thereby defining a important epitope for the antibody. Human monoclonal antibodies having these properties are particularly useful at neutralizing field isolates, and therefore provide useful information regarding the immunocompetence of an immune response in HIV-infected patients.

Therefore, the invention provides for a screening method to determine whether HIV-infected patients contain antibodies of the class that neutralize field isolates. The method for determining immunocompetence of a human anti-human immunodeficiency virus (HIV) antibody in a sample comprises the steps of:

(1) contacting a sample believed to contain a human anti-HIV antibody with a diagnostically effective amount of the above-described anti-gp120 monoclonal antibody in a competition immunoreaction admixture containing mature gp120 in the solid phase;

(2) maintaining the competition immunoreaction admixture under conditions sufficient for the monoclonal antibody to bind with the gp120 in the solid phase and form a solid phase immunoreactant; and

(3) detecting the amount of the immunoreactant present in the solid phase, and thereby the immunocompetence of any human anti-HIV antibody in the

sample.

Another preferred human monoclonal antibody has the binding specificity of a monoclonal antibody comprising a light chain immunoglobulin variable region amino acid residue sequence selected from the group consisting of SEQ ID Nos 95, 96, 97, 98, 101, 102, 103, 104, 105, 107, 110, 115, 118, 121, 122, 124 and 132.

In a further embodiment, the invention contemplates a human monoclonal antibody capable of immunoreacting with human immunodeficiency virus (HIV) glycoprotein gp41 and neutralizing HIV. A preferred human monoclonal antibody has the binding specificity of a monoclonal antibody comprising a heavy chain immunoglobulin variable region amino acid residue sequence selected from the group consisting of SEQ ID Nos 142, 143, 144, 145 and 146. Another preferred human monoclonal antibody has the binding specificity of a monoclonal antibody comprising a light chain immunoglobulin variable region amino acid residue sequence selected from the group consisting of SEQ ID Nos 147, 148, 149, 150 and 151.

In another embodiment, the invention describes a polynucleotide sequence encoding a heavy or light chain immunoglobulin variable region amino acid residue sequence portion of a human monoclonal antibody of this invention. Also contemplated are DNA expression vectors containing the polynucleotide, and host cells containing the vectors and polynucleotides of the invention.

The invention also contemplates a method of detecting human immunodeficiency virus (HIV) comprising contacting a sample suspected of containing HIV with a diagnostically effective amount of the monoclonal antibody of this invention, and determining whether the monoclonal antibody immunoreacts with the sample. The method can be practiced in vitro or in vivo, and may include a variety of methods for determining the presence of an immunoreaction product.

In another embodiment, the invention describes a method for providing passive immunotherapy to human immunodeficiency virus (HIV) disease in a human,

comprising administering to the human an immunotherapeutically effective amount of the monoclonal antibody of this invention. The administration can be provided prophylactically, and by a parenteral administration. Pharmaceutical compositions containing one or more of the different human monoclonal antibodies are described for use in the therapeutic methods of the invention.

10 Brief Description of the Drawings

In the drawings forming a portion of this disclosure: Figure 1 illustrates the sequence of the double-stranded synthetic DNA inserted into Lambda Zap to produce a Lambda Hc2 expression vector. The preparation of the double-stranded synthetic DNA insert is described in Example 1a2). The various features required for this vector to express the V_H -coding DNA homologs include the Shine-Dalgarno ribosome binding site, a leader sequence to direct the expressed protein to the periplasm as described by Mouva et al., J. Biol. Chem., 255:27, 1980, and various restriction enzyme sites used to operatively link the V_H homologs to the expression vector. The V_H expression vector sequence also contains a short nucleic acid sequence that codes for amino acids typically found in variable regions heavy chain (V_H backbone). This V_H backbone is just upstream and in the proper reading as the V_H DNA homologs that are operatively linked into the Xho I and Spe I cloning sites. The sequences of the top and bottom strands of the double-stranded synthetic DNA insert are listed respectively in SEQ ID NO 1 and SEQ ID NO 2. The ten amino acid sequence comprising the decapeptide tag is listed in SEQ ID NO 5. The synthetic DNA insert is directionally ligated into Lambda Zap II digested with the restriction enzymes Not I and Xho I to form Lambda Hc2 expression vector.

Figure 2 illustrates the major features of the bacterial expression vector Lambda Hc2 (V_H expression vector). The orientation of the insert in Lambda Zap II

is shown. The V_H DNA homologs are inserted into the Xho I and Spe I cloning sites. The read through transcription produces the decapeptide epitope (tag) that is located just 3' of the cloning site. The amino acid residue sequence of the decapeptide tag and the Pel B leader sequence/spacer are respectively listed in SEQ ID NO 5 and 6.

Figure 3 illustrates the sequence of the double-stranded synthetic DNA inserted into Lambda Zap to produce a Lambda Lc2 expression vector. The various features required for this vector to express the V_L -coding DNA homologs are described in Figure 1. The V_L -coding DNA homologs are operatively linked into the Lc2 sequence at the Sac I and Xho I restriction sites. The sequences of the top and bottom strands of the double-stranded synthetic DNA insert are listed respectively in SEQ ID NO 3 and SEQ ID NO 4. The synthetic DNA insert is directionally ligated into Lambda Zap II digested with the restriction enzymes Sac I and Not I to form Lambda Lc2 expression vector.

Figure 4 illustrates the major features of the bacterial expression vector Lc2 (V_L expression vector). The synthetic DNA sequence from Figure 3 is shown at the top along with the LacZ promoter from Lambda Zap II. The orientation of the insert in Lambda Zap II is shown. The V_L DNA homologs are inserted into the Sac I and Xho I cloning sites. The amino acid residue sequence of the Pel B leader sequence/spacer is listed in SEQ ID NO 7.

Figure 5 illustrates the dicistronic expression vector, pComb, in the form of a phagemid expression vector.

Figure 6 illustrates the neutralization of HIV-1 by recombinant Fabs. The same supernate preparations were used in p24 and syncytia assays. The figures indicate neutralization titers. Refer to Example 3 for details of the assay procedures and discussion of the results. The ELISA titers and Fab concentrations were determined as described in Example 2b.

Figure 7 illustrates the relative affinities of Fab fragments for gp120 (IIIB) as illustrated by inhibition ELISA performed as described in Example 2b6). Fabs 27, 6, 29, 2 and 3 are all prototype members of the different groups discussed in Example 9a. Loop 2 is an Fab fragment selected from the same library as the other Fabs but which recognizes the V3 loop. The data is plotted as the percentage of maximum binding on the Y-axis against increasing concentrations (10^{-11} M to 10^{-7} M) of soluble gp120 on the X-axis.

Figure 8 illustrates the soluble CD4 competition with Fab fragments for gp120 (IIIB). P4D10 and loop2 are controls. P4D10 is a mouse monoclonal antibody reacting with the V3 loop of gp120 (IIIB). The data, discussed in Example 2b6), is plotted as described in Figure 7.

Figure 9 illustrates the neutralization of HIV by purified Fabs prepared as described in Example 3. The results shown are derived from the syncytia assay using the MN strain. The data is plotted as percent of inhibition of binding on the Y-axis against increasing Fab concentrations [0.1 to greater than 10 micrograms/milliliter ($\mu\text{g/ml}$)] on the X-axis.

Figures 10A and 10B illustrate the amino acid residue sequences of variable heavy (V_H) domains of Fabs binding to gp120. Seven distinct groups have been identified as described in Example 9a based on sequence homology. Identity with the first sequence in a group is indicated by dots. The Fab clone names are indicated in the left hand column. The corresponding SEQ ID Nos are indicated in the right hand column. The sequenced regions from right to left are framework region 1 (FR1), complementary determining region 1 (CDR1), framework region 2 (FR2), complementary determining region 2 (CDR2), framework region 3 (FR3), complementary determining region 3 (CDR3), and framework region 4 (FR4). The five amino-terminal residue sequence beginning with LEQ arises from the VH1a while the 5 amino-terminal residue sequence beginning with LEE arises from the VH3a primers. The b11 and b29

sequences are very similar to the b3 group and could be argued to be intracloonal variants within that group; they are placed in their own group because of differences at the V-D and D-J interface.

5 Figures 11A and 11B illustrate the amino acid residue sequences of variable light (V_L) domains of Fabs binding to gp120. Refer to Figures 10A and 10B for the description of the figure and to Example 9b for analysis of the sequences.

10 Figures 12A and 12B illustrate the amino acid residue sequences of V_L domains from Fabs binding to gp120 and generated by shuffling the heavy chain from clone b12 against a library of light chains (H12-LCn Fabs) as described in Example 10. Note that the new V_L sequences
15 have designated clone numbers that do not relate to those numbers from the original library. The unique sequences are listed in the Sequence Listing from SEQ ID NO 114 to 122. The new V_L domain sequences are compared to that of the original clone b12 V_L sequence.

20 Figures 13A and 13B illustrate the amino acid residue sequences of V_H domains from Fabs binding to gp120 and generated by shuffling the light chain from clone b12 against a library of heavy chains (L12-HCn Fabs) as described in Example 10. Note that the new V_H sequences
25 have designated clone numbers that do not relate to those numbers from the original library. The unique sequences are listed in the Sequence Listing from SEQ ID NO 123 to 132. The new V_H domain sequences are compared to that of the original clone b12 V_H sequence.

30 Figures 14A and 14B illustrate plasmid maps of the heavy (pTAC01H) and light chain (pTC01) replicon-compatible chain-shuffling vectors, respectively. Both plasmids are very similar in the section containing the promoter and the cloning site. Abbreviations: tacPO,
35 tac promoter/operon; 5 histidine amino acid residue tag (histidine)5-tail; flIG, intergenic region of fl-phage; stu, stuffer fragment ready for in-frame replacement by light and heavy chain, respectively; cat, chloramphenicol

transferase gene; bla, b-lactamase gene; ori, origin of replication. The map is drawn approximately to scale.

5 Figures 15A and 15B illustrate the nucleotide sequences of the binary shuffling vectors. The construction and use of the vectors is described in Example 11. In Figure 15A, the double-stranded nucleotide sequence of the multiple cloning site in light chain vector, pTC01, is shown. The sequences of the top and bottom nucleotide base strands are listed respectively in SEQ ID NO 8 and SEQ ID NO 9.

10 The amino acid residue sequence comprising the pelB leader ending in the Sac I restriction site is listed in SEQ ID NO 10. In Figure 15B, the nucleotide sequence of the multiple cloning site in heavy chain vector, pTAC01H, is shown. The sequences of the top and bottom nucleotide

15 base strands are listed respectively in SEQ ID NO 11 and SEQ ID NO 12. The amino acid residue sequence comprising the pelB leader ending in the Xho I restriction site is listed as SEQ ID NO 13. The amino acid residue sequence comprising the histidine tail is listed in SEQ ID NO 14.

20 Relevant restriction sites are underlined. tac promoter and ribosome binding site (rbs) are indicated by boxes.

Figure 16 illustrates the complete set of directed crosses between heavy and light chains of all Fab fragments isolated from the original library by panning

25 with gp160 (IIIB) (b1-b27), gp120 (IIIB) (B8-B35), gp120 (SF2) (s4-s8), and the loop peptide (p35) assayed by ELISA against IIIB gp120 as described in Example 11. Heavy chains are listed horizontally and light chains are listed vertically. Clones are sorted according to the grouping

30 established in Example 9. Different groups are separated by horizontal and vertical lines. A "-" at the intersection of a particular heavy chain and light chain signifies a clear negative (a signal of 3 times background or less) for that particular cross, a "+" shows a clear

35 positive comparable to the original heavy and light chain combination, and a "w" denotes an intermediate value in the ELISA. "●": the HCp35/ LCp35 combination is negative when gp120 (IIIB) is used, but positive when assayed with

gp120 (IIIB). Identical chains carry the same identifier (either *, ¶, §, or ¥).

Figure 17 illustrates the affinity of antibody-antigen interaction for b12 heavy chain crosses with light chains from all pannings analyzed by competitive ELISA using soluble IIIB gp120 as competing antigen as described in Example 10. The data is plotted as the percentage of maximum binding on the Y-axis against increasing concentrations of soluble gp120 (IIIB) (10^{-12} M to 10^{-7} M) on the X-axis.

Figures 18A and 18B illustrate the amino acid residue sequences of variable heavy (V_H) domains of Fabs binding to gp41. The Fab clone names are indicated in the left hand column. The heavy chain sequences of the five Fabs individually designated DL 41 19, DO 41 11, GL 41 1, MT 41 12 and SS 41 8 have been assigned the respective SEQ ID Nos 142, 143, 144, 145 and 146. The sequenced regions from right to left are framework region 1 (FR1), complementary determining region 1 (CDR1), framework region 2 (FR2), complementary determining region 2 (CDR2), framework region 3 (FR3), complementary determining region 3 (CDR3), and framework region 4 (FR4).

Figures 19A and 19B illustrate the amino acid residue sequences of variable light (V_L) domains of Fabs binding to gp41. Refer to Figures 18A and 18B for the description of the figure. The light chain sequences of the five Fabs individually designated DL 41 19, DO 41 11, GL 41 1, MT 41 12 and SS 41 8 have been assigned the respective SEQ ID NOs 147, 148, 149, 150 and 151.

Figure 20 illustrates the relative binding affinities of b3, b6, and b12 for the total envelope glycoproteins (gp160) and for the gp120 glycoprotein (gp120) expressed on the surface of COS-1 cells as determined by immunoprecipitation and described in Example 6. The signal on the autoradiogram represents the relative amount of envelope glycoproteins bound with increasing concentrations of Fab (0-150 μ g/ml).

Figure 21 illustrates the neutralization of HIV-1 by b12 IgG1 as assessed using PHA-stimulated PBMCs as indicator cells and determination of extracellular p24 as the reporter assay. Refer to Example 5d for details of the assay procedures and discussion of the results. The designation, location, and disease status of the virus donors were as follows: ■, VS (New York, acute), ▼, N70-2 (New Orleans, asymptomatic), ▲, AC (San Diego, AIDS), ●, LS (Los Angeles, AIDS), □, NYC-A (New York, unknown), ∇, WM (Los Angeles, AIDS), △, RA (New York, acute), ◇, JP (New York, acute). The molecularly cloned HIV-1 virus JR-CSF (◆) and HIV-1 isolate JR-FL (○) were also assayed for neutralization. The data is plotted as % neutralization on the Y-axis against increasing concentrations of b12 IgG1 (0-25 µg/ml) on the X-axis.

Figure 22 illustrates the reactivity of b12 IgG1 with a panel of international isolates of HIV-1 as described in Example 8. Reactivity was determined with gp120 isolated from the HIV-1 samples in ELISA with the b12 IgG1 as described in Example 8. Data is plotted as % b12 IgG1 reactivity on the X-axis against clades A-F on the Y-axis. Country names indicate where the HIV-1 virus was originally isolated. The numbers in parenthesis refer to the number of viruses of each clade examined. Reactivity is designated as strong (⊗) or moderate (⊘).

Figure 23 illustrates the neutralization of the HXBc2 molecular clone of HIV-1 LAI by purified Fabs and a monoclonal antibody 110.4 (Mab 110.4) in an envelope complementation assay as described in Example 3c. Neutralization of HXBc2 infectivity is expressed as a decrease in residual CAT activity. The data is plotted as % residual CAT activity on the Y-axis and increasing concentrations of Fab and MAb (0.1-20 µg/ml) on the X-axis.

Figure 24 illustrates the pSG-5 mammalian expression vector as described in Examples 4a and 4b. Transcription of the heavy or light chain gene when inserted in the EcoRI site is under the control of the SV40 early

promoter. Transcriptional termination is signaled by the SV40 polyadenylation signal sequence downstream of the heavy chain sequence. The M13 intergenic region allows for the production of single-stranded DNA for nucleotide sequence determination. The amp^R gene is for selection of the vector in bacterial cells.

Figures 25A and 25B illustrate the nucleotide and amino acid residue sequences of the b12 light chain gene in the pSG-5 mammalian expression vector described in Example 4b. The b12 light chain has been modified for expression in mammalian cells as described in Example 4b.

Figure 26 illustrates pEe6HC BM12, the pEE6 mammalian expression vector with the b12 IgG1 heavy chain gene that has been modified for antibody expression in mammalian cells as described in Example 4d. The VH was originally derived from the Fab b12 and has the same binding specificity as the Fab b12. The pEE6 vector has a human CMV promoter for expression of the heavy chain, a polyadenylation signal for termination of transcription, and an ampicillin gene for selection in bacteria.

Figures 27A through 27E illustrate the nucleotide sequences of the b12 heavy chain VH and constant regions in the pEe6HC BM12 mammalian expression vector as described Example 4d. The amino acid residue sequence of the b12 heavy chain VH is given. The b12 VH has been modified for expression in mammalian cells as described in Example 4d.

Figure 28 illustrates pEe12 Combo BM12, the pEE12 mammalian expression vector with b12 IgG1 heavy and light chain genes that have been modified for antibody expression in mammalian cells as described in example 4f. The VH and light chain were originally derived from the Fab b12 and have the same binding specificity as the Fab b12. The pEE12 vector has a human CMV promoter for expression of the light chain, a polylinker to provide cloning sites, and a polyadenylation signal for termination of transcription. The vector also contains the GS selectable marker gene whose expression is

controlled an SV40 early promoter at the 5' end of the GS gene, an intron, and a polyadenylation signal at the 3' end of the GS gene. A heavy chain cassette comprising the HCMV promoter, enhancer elements, heavy chain gene, and polyadenylation signal were removed from the pEE6 vector and inserted into the pEE12 vector to generate the combinatorial construct containing both the b12 light and heavy chain genes.

Figure 29A through 29R illustrates the nucleotide sequence of the pEE12 mammalian expression vector and the b12 IgG1 heavy and light chain genes, pEel2 Combo BM 12, as described in Example 4f. The VH and light chain genes have been modified for expression in mammalian cells as described in Example 4.

Detailed Description of the Invention

A. Definitions

Amino Acid Residue: An amino acid formed upon chemical digestion (hydrolysis) of a polypeptide at its peptide linkages. The amino acid residues described herein are preferably in the "L" isomeric form. However, residues in the "D" isomeric form can be substituted for any L-amino acid residue, as long as the desired functional property is retained by the polypeptide. NH₂ refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxy terminus of a polypeptide. In keeping with standard polypeptide nomenclature (described in J. Biol. Chem., 243:3552-59 (1969) and adopted at 37 CFR §1.822(b)(2)), abbreviations for amino acid residues are shown in the following Table of Correspondence:

TABLE OF CORRESPONDENCE

<u>SYMBOL</u>		<u>AMINO ACID</u>
<u>1-Letter</u>	<u>3-Letter</u>	
Y	Tyr	tyrosine
G	Gly	glycine

	F	Phe	phenylalanine
	M	Met	methionine
	A	Ala	alanine
	S	Ser	serine
5	I	Ile	isoleucine
	L	Leu	leucine
	T	Thr	threonine
	V	Val	valine
	P	Pro	proline
10	K	Lys	lysine
	H	His	histidine
	Q	Gln	glutamine
	E	Glu	glutamic acid
	Z	Glx	Glu and/or Gln
15	W	Trp	tryptophan
	R	Arg	arginine
	D	Asp	aspartic acid
	N	Asn	asparagine
	B	Asx	Asn and/or Asp
20	C	Cys	cysteine
	X	Xaa	Unknown or other

It should be noted that all amino acid residue sequences represented herein by formulae have a left- to-right orientation in the conventional direction of amino terminus to carboxy terminus. In addition, the phrase "amino acid residue" is broadly defined to include the amino acids listed in the Table of Correspondence and modified and unusual amino acids, such as those listed in 37 CFR 1.822(b)(4), and incorporated herein by reference. Furthermore, it should be noted that a dash at the beginning or end of an amino acid residue sequence indicates a peptide bond to a further sequence of one or more amino acid residues or a covalent bond to an amino-terminal group such as NH₂ or acetyl or to a carboxy-terminal group such as COOH.

Recombinant DNA (rDNA) molecule: A DNA molecule produced by operatively linking two DNA segments. Thus, a recombinant DNA molecule is a hybrid DNA molecule

comprising at least two nucleotide sequences not normally found together in nature. RDNA'S not having a common biological origin, i.e., evolutionarily different, are said to be "heterologous".

5 Vector: A RDNA molecule capable of autonomous replication in a cell and to which a DNA segment, e.g., gene or polynucleotide, can be operatively linked so as to bring about replication of the attached segment. Vectors
10 capable of directing the expression of genes encoding for one or more polypeptides are referred to herein as "expression vectors". Particularly important vectors allow cloning of cDNA (complementary DNA) from mRNAs produced using reverse transcriptase.

Receptor: A receptor is a molecule, such as a
15 protein, glycoprotein and the like, that can specifically (non-randomly) bind to another molecule.

Antibody: The term antibody in its various grammatical forms is used herein to refer to
20 immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antibody combining site or paratope. Exemplary antibody molecules are intact immunoglobulin molecules, substantially intact immunoglobulin molecules and portions of an immunoglobulin molecule, including those portions
25 known in the art as Fab, Fab', F(ab')₂ and F(v).

Antibody Combining Site: An antibody combining site is that structural portion of an antibody molecule comprised of a heavy and light chain variable and hypervariable regions that specifically binds
30 (immunoreacts with) an antigen. The term immunoreact in its various forms means specific binding between an antigenic determinant-containing molecule and a molecule containing an antibody combining site such as a whole antibody molecule or a portion thereof.

35 Monoclonal Antibody: A monoclonal antibody in its various grammatical forms refers to a population of antibody molecules that contain only one species of antibody combining site capable of immunoreacting with a

particular epitope. A monoclonal antibody thus typically displays a single binding affinity for any epitope with which it immunoreacts. A monoclonal antibody may therefore contain an antibody molecule having a plurality of antibody combining sites, each immunospecific for a different epitope, e.g., a bispecific monoclonal antibody. Although historically a monoclonal antibody was produced by immortalization of a clonally pure immunoglobulin secreting cell line, a monoclonally pure population of antibody molecules can also be prepared by the methods of the present invention.

Fusion Polypeptide: A polypeptide comprised of at least two polypeptides and a linking sequence to operatively link the two polypeptides into one continuous polypeptide. The two polypeptides linked in a fusion polypeptide are typically derived from two independent sources, and therefore a fusion polypeptide comprises two linked polypeptides not normally found linked in nature.

Upstream: In the direction opposite to the direction of DNA transcription, and therefore going from 5' to 3' on the non-coding strand, or 3' to 5' on the mRNA.

Downstream: Further along a DNA sequence in the direction of sequence transcription or read out, that is traveling in a 3'- to 5'-direction along the non-coding strand of the DNA or 5'- to 3'-direction along the RNA transcript.

Cistron: Sequence of nucleotides in a DNA molecule coding for an amino acid residue sequence and including upstream and downstream DNA expression control elements.

Leader Polypeptide: A short length of amino acid sequence at the amino end of a polypeptide, which carries or directs the polypeptide through the inner membrane and so ensures its eventual secretion into the periplasmic space and perhaps beyond. The leader sequence peptide is commonly removed before the polypeptide becomes active.

Reading Frame: Particular sequence of contiguous

nucleotide triplets (codons) employed in translation. The reading frame depends on the location of the translation initiation codon.

5

B. Human Monoclonal Antibodies

The present invention relates to human monoclonal antibodies which are specific for, and neutralize human immunodeficiency virus (HIV). In a preferred embodiment of the invention, human monoclonal antibodies are disclosed which are capable of binding epitopic polypeptide sequences in glycoprotein gp120 of HIV. A further preferred embodiment are human monoclonal antibodies capable of binding epitopic polypeptide sequences in glycoprotein gp 41 of HIV. Also disclosed is an antibody having a specified amino acid sequence, which sequence confers the ability to bind a specific epitope and to neutralize HIV when the virus is bound by these antibodies. A human monoclonal antibody with a claimed specificity, and like human monoclonal antibodies with like specificity, are useful in the diagnosis and immunotherapy of HIV-induced disease.

The term "HIV-induced disease" means any disease caused, directly or indirectly, by HIV. An example of a HIV-induced disease is acquired autoimmunodeficiency syndrome (AIDS), and any of the numerous conditions associated generally with AIDS which are caused by HIV infection.

Thus, in one aspect, the present invention is directed to human monoclonal antibodies which are reactive with a HIV neutralization site and cell lines which produce such antibodies. The isolation of cell lines producing monoclonal antibodies of the invention is described in great detail further herein, and can be accomplished using the phagemid vector library methods described herein, and using routine screening techniques which permit determination of the elementary immunoreaction and neutralization patterns of the monoclonal antibody of interest. Thus, if a human

monoclonal antibody being tested binds and neutralizes HIV in a manner similar to a human monoclonal antibody produced by the cell lines of the invention then the tested antibody is considered equivalent to an antibody of the invention.

It is also possible to determine, without undue experimentation, if a human monoclonal antibody has the same (i.e., equivalent) specificity as a human monoclonal antibody of this invention by ascertaining whether the former prevents the latter from binding to HIV. If the human monoclonal antibody being tested competes with the human monoclonal antibody of the invention, as shown by a decrease in binding by the human monoclonal antibody of the invention in standard competition assays for binding to a solid phase antigen, for example to gp120, then it is likely that the two monoclonal antibodies bind to the same, or a closely related, epitope.

Still another way to determine whether a human monoclonal antibody has the specificity of a human monoclonal antibody of the invention is to pre-incubate the human monoclonal antibody of the invention with HIV with which it is normally reactive, and then add the human monoclonal antibody being tested to determine if the human monoclonal antibody being tested is inhibited in its ability to bind HIV. If the human monoclonal antibody being tested is inhibited then, in all likelihood, it has the same, or functionally equivalent, epitopic specificity as the monoclonal antibody of the invention. Screening of human monoclonal antibodies of the invention, can be also carried out utilizing HIV neutralization assays and determining whether the monoclonal antibody neutralizes HIV.

The ability to neutralize HIV at one or more stages of virus infection is a desirable quality of a human monoclonal antibody of the present invention. Virus neutralization can be measured by a variety of in vitro and in vivo methodologies. Exemplary methods described herein for determining the capacity for neutralization are

the in vitro assays that measure inhibition of HIV-induced syncytia formation, plaque assays and assays that measure the inhibition of output of core p24 antigen from a cell infected with HIV.

5 As shown herein, the immunospecificity of a human monoclonal antibody of this invention can be directed to epitopes that are shared across serotypes and/or strains of HIV, or can be specific for a single strain of HIV, depending upon the epitope. Thus, a preferred human
10 monoclonal antibody can immunoreact with HIV-1, HIV-2, or both, and can immunoreact with one or more of the HIV-1 strains IIIB, MN, RF, SF-2, Z2, Z6, CDC4, ELI and the like strains. In addition, a preferred human monoclonal
15 antibody can immunoreact and neutralize a majority of field isolates of HIV, as described further herein.

 The immunospecificity of an antibody, its HIV-neutralizing capacity, and the attendant affinity the antibody exhibits for the epitope, are defined by the epitope with which the antibody immunoreacts. The epitope
20 specificity is defined at least in part by the amino acid residue sequence of the variable region of the heavy chain of the immunoglobulin the antibody, and in part by the light chain variable region amino acid residue sequence. Preferred human monoclonal antibodies immunoreact with the
25 CD4 binding site of glycoprotein gp120.

 Also disclosed is an antibody having a specified amino acid sequence, which sequence confers the ability to bind a specific unique neutralizing epitope and to
30 neutralize HIV when the virus is bound by these antibodies.

 A preferred human monoclonal antibody of this invention has the binding specificity of a monoclonal antibody comprising a heavy chain immunoglobulin variable region amino acid residue sequence selected from the group
35 of sequences consisting of SEQ ID NOs 66, 67, 68, 70, 72, 73, 74, 75, 78 and 97, and conservative substitutions thereof.

Another preferred human monoclonal antibody of this invention has the binding specificity of a monoclonal antibody having a light chain immunoglobulin variable region amino acid residue sequence selected from the group of sequences consisting of SEQ ID NOs 95, 96, 97, 98, 101, 102, 103, 104, 105, 107, 110, 115, 118, 121, 122, 124 and 132, and conservative substitutions thereof.

In a preferred embodiment, a monoclonal antibodies of this invention exhibits a potent capacity to neutralize HIV. The capacity to neutralize HIV is expressed as a concentration of antibody molecules required to reduce the infectivity titer of a suspension of HIV when assayed in an typical in vitro infectivity assay, such as is described herein. A monoclonal antibody of this invention has the capacity to reduce HIV infectivity titer in an in vitro virus infectivity assay by 50% at a concentration of less than 700 nanograms (ng) of antibody per milliliter (ml) of culture medium in the assay, and preferably reduces infectivity titers 50% at a concentration of less than 300 ng/ml, and more preferably at concentrations less than about 10 ng/ml.

Exemplary and preferred monoclonal antibodies described herein are effective at 3-700 ng/ml, and therefore are particularly well suited for inhibiting HIV in vitro and in vivo.

Particularly preferred human monoclonal antibodies of this invention immunoreact with gp120 in its "mature" form, which form is to be distinguished from antigenic determinants present on the HIV envelope precursor glycoprotein designated gp160. gp160 is processed during virus biogenesis by cleavage into two polypeptides, gp41 and gp120. "Mature" gp120 refers to the processed protein that is found in mature HIV virus particles, and can be detected on the surface of HIV-infected cells.

Thus, a preferred antibody of this invention binds mature gp120 preferentially over HIV precursor glycoprotein gp160. By "binds preferentially" is meant that the antibody immunoreacts with (binds) substantially

more mature gp120 than gp160 in an immunoreaction admixture. Substantially more typically indicates that at least greater than 50 % of the total mass of immunoprecipitated material is gp120, and preferably indicates that at least greater than 75 %, more preferably 90 %, of the immunoprecipitated material is gp120.

Methods for determining immunoreaction of a subject antibody with gp120 or gp160 are well known in the art, and the invention need not be so limited. However, preferred methods for determining the relative amounts of envelope glycoprotein antigens are described in the Examples, and include radio-immunoprecipitation (RIP) of cell-surface labeled HIV-infected cells, followed by molecular weight analysis of the labeled products by polyacrylamide gel electrophoresis (PAGE).

A preferred human monoclonal antibody also has the ability to immunoreact with native gp120 and comparatively bind substantially less of a variant gp120 produced by recombinant DNA methods in which the V1 and V2 loops have been deleted. The variant gp120, also referred to a V1/V2 loop deficient-variant gp120, is described in the Examples, and is seen to bind substantially less of a preferred antibody, b12, in comparison to native gp120. The term "native gp120" refers to a mature gp120 protein having a normal amino acid residue sequence instead of a variant protein having selected amino acid residue substitutions or deletions, such as the V1/V2 loop deficient-variant in which the V1 and V2 loops were deleted. This preferential binding with native gp120 compared to the V1/V2 loop deficient-variant identifies an important epitope defined by a preferred antibody of this invention. Antibodies having this binding epitope are particularly effective at neutralizing a majority of field isolates of HIV, as described herein.

The ability to bind "substantially less" V1/V2 loop deficient-variant gp120 than native gp120 can be readily measured using various immunoreaction detection methods, although the assay methods described in Example

5c are particularly preferred. In preferred embodiments, substantially less binding to V1/V2 loop deficient-variant gp120 compared to native gp120 is indicated when the comparison is conducted as described as in Example 5c, and the native gp120 exhibits a ratio value deviating from the mean of greater than 2.0 and the variant exhibits a ratio value deviating from the mean of less than 0.5.

A particularly preferred human monoclonal antibody of this invention also has the capacity to neutralize a majority of field isolates as disclosed herein. As is well understood, the field (i.e., clinically isolated) strains of HIV are typically different to some degree antigenically from laboratory strains. Therefor, it is well understood that useful neutralizing antibodies must immunoreact with, and be neutralizing against, field isolates of HIV. Preferably, the useful antibody neutralized a large percentage of field isolates, thereby increasing its effectiveness when new strains are encountered.

The Examples demonstrate that the human monoclonal antibody b12 has the ability to neutralize a majority of the field isolates tested. By majority is meant that in a representative and diverse collection of field isolates, the antibody is capable of neutralizing at least 50 % of the strains, and preferably at least 75 % of the strains tested. In this context, "neutralizing" means an effect of reducing the HIV infectivity titre in an in vitro virus infectivity assay as described herein at the antibody concentrations described.

Thus, the invention also contemplates a human monoclonal antibody capable of immunoreacting with and neutralizing a first preselected human immunodeficiency virus (HIV), such as the laboratory isolate MN or IIIB, that is further capable of immunoreacting with and neutralizing one or more other (i.e., second) strains of HIV, particularly field strains. In this embodiment, supported by the teachings of the Examples, the antibody has the capacity to reduce HIV infectivity titer in an in

vitro virus infectivity assay of the first HIV strain by 50% at a concentration of at least less than 700 nanograms (ng) of antibody per milliliter (ml), and has the capacity to reduce HIV infectivity titer of a second field strain of HIV in the same in vitro virus infectivity assay by 50% at a concentration of less than about 700 nanograms (ng) of antibody per milliliter (ml). In more preferred embodiments and depending upon the particular HIV strain, the capacity to reduce second field strain infectivity titers by 50% can be exhibited at lower antibody concentrations, such as below 300 ng/ml.

A particularly preferred antibody is an antibody having the binding specificity of the b12 monoclonal antibody described herein. The amino acid residue sequence of the heavy chain variable region of b12 is shown in SEQ ID NO 66, and the light chain variable region sequence of b12 is shown in SEQ ID NO 97. Still more preferred are human antibodies having the binding specificity of the immunoglobulin heavy and light chain polypeptides produced by ATCC 69079.

Further preferred human monoclonal antibodies immunoreact with the CD4 binding site of glycoprotein gp41. A preferred human monoclonal antibody of this invention has the binding specificity of a monoclonal antibody comprising a heavy chain immunoglobulin variable region amino acid residue sequence selected from the group of sequences consisting of SEQ ID NOs 142, 143, 144, 145, and 146 and conservative substitutions thereof.

Another preferred human monoclonal antibody of this invention has the gp41 binding specificity of a monoclonal antibody having a light chain immunoglobulin variable region amino acid residue sequence selected from the group of sequences consisting of SEQ ID NOs 147, 148, 149, 150, and 151 and conservative substitutions thereof.

As shown by the present teachings and using the combinatorial library shuffling and screening methods, one can identify new heavy and light chain pairs (H:L) that function as a HIV-neutralizing monoclonal antibody. In

particular, one can shuffle a known heavy chain, derived from an HIV-neutralizing human monoclonal antibody, with a library of light chains to identify new H:L pairs that form a functional antibody according to the present invention. Similarly, one can shuffle a known light chain, derived from an HIV-neutralizing human monoclonal antibody, with a library of heavy chains to identify new H:L pairs that form a functional antibody according to the present invention.

Particularly preferred human monoclonal antibodies are those having the gp120 immunoreaction (binding) specificity of a monoclonal antibody having heavy and light chain immunoglobulin variable region amino acid residue sequences in pairs (H:L) selected from the group consisting of SEQ ID NOs 66:95, 67:96, 72:102, 66:97, 73:107, 74:103, 70:101, 68:98, 75:104, 72:105, 78:110, 66:118, 66:122, 66:121, 66:115, 97:124, 97:132 and 66:98, and conservative substitutions thereof. The designation of two SEQ ID NOs with a colon, e.g., 66:95, is to connote a H:L pair formed by the heavy and light chain, respectively, amino acid residue sequences shown in SEQ ID NO 66 and SEQ ID NO 95, respectively.

Further preferred human monoclonal antibodies are those having the gp41 immunoreaction (binding) specificity of a monoclonal antibody having heavy and light chain immunoglobulin variable region amino acid residue sequences in pairs (H:L) selected from the group consisting of SEQ ID NOs 142:147, 143:148, 144:149, 145:150, and 146:151, and conservative substitutions thereof.

Particularly preferred are human monoclonal antibodies having the binding specificity of the monoclonal antibody produced by the E. coli microorganisms deposited with the ATCC, as described further herein.

Particularly preferred are human monoclonal antibodies having the binding specificity of the monoclonal antibodies produced by the E. coli microorganisms designated ATCC 69078, 69079 and 69080. By

"having the binding specificity" is meant equivalent monoclonal antibodies which exhibit the same or similar immunoreaction and neutralization properties, and which compete for binding to an HIV antigen. Preferred are the human monoclonal antibodies produced by ATCC 69078, 69079 and 69080.

The term "conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, and the like. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies having the substituted polypeptide also neutralize HIV. Analogously, another preferred embodiment of the invention relates to polynucleotides which encode the above noted heavy and/or light chain polypeptides and to polynucleotide sequences which are complementary to these polynucleotide sequences. Complementary polynucleotide sequences include those sequences which hybridize to the polynucleotide sequences of the invention under stringent hybridization conditions.

By using the human monoclonal antibodies of the invention, it is now possible to produce anti-idiotypic antibodies which can be used to screen human monoclonal antibodies to identify whether the antibody has the same binding specificity as a human monoclonal antibody of the invention and also used for active immunization (Herlyn et al., Science, 232:100 (1986)). Such anti-idiotypic antibodies can be produced using well-known hybridoma techniques (Kohler et al., Nature, 256:495 (1975)). An anti-idiotypic antibody is an antibody which recognizes unique determinants present on the human monoclonal antibody produced by the cell line of interest. These

determinants are located in the hypervariable region of the antibody. It is this region which binds to a given epitope and, thus, is responsible for the specificity of the antibody. An anti-idiotypic antibody can be prepared
5 by immunizing an animal with the monoclonal antibody of interest. The immunized animal will recognize and respond to the idiotypic determinants of the immunizing antibody and produce an antibody to these idiotypic determinants. By using the anti-idiotypic antibodies of the immunized
10 animal, which are specific for the human monoclonal antibody of the invention produced by a cell line which was used to immunize the second animal, it is now possible to identify other clones with the same idio type as the antibody of the hybridoma used for immunization.
15 Idiotypic identity between human monoclonal antibodies of two cell lines demonstrates that the two monoclonal antibodies are the same with respect to their recognition of the same epitopic determinant. Thus, by using anti-idiotypic antibodies, it is possible to identify other
20 hybridomas expressing monoclonal antibodies having the same epitopic specificity.

It is also possible to use the anti-idiotypic technology to produce monoclonal antibodies which mimic an epitope. For example, an anti-idiotypic monoclonal
25 antibody made to a first monoclonal antibody will have a binding domain in the hypervariable region which is the "image" of the epitope bound by the first monoclonal antibody. Thus, the anti-idiotypic monoclonal antibody can be used for immunization, since the anti-idiotypic
30 monoclonal antibody binding domain effectively acts as an antigen.

In one preferred embodiment, the invention contemplates a truncated immunoglobulin molecule comprising a Fab fragment derived from a human monoclonal
35 antibody of this invention. The Fab fragment, lacking Fc receptor, is soluble, and affords therapeutic advantages in serum half life, and diagnostic advantages in modes of using the soluble Fab fragment. The preparation of a

soluble Fab fragment is generally known in the immunological arts and can be accomplished by a variety of methods. A preferred method of producing a soluble Fab fragment is described herein.

5 In another preferred embodiment, the invention contemplates an immunoglobulin molecule comprising a Fab fragment derived from a human monoclonal antibody of this invention and the fragment crystallizable (Fc) domain of a human immunoglobulin molecule. The entire (i.e.,
10 complete) immunoglobulin (Ig) molecule comprising a Fab fragment with the Fc domain may afford therapeutic and diagnostic advantages, and can be any of the several Ig species depending upon the ultimate use, including IgG, IgA, IgD, IgE, IgM, and isotypes thereof. The
15 immunoglobulin molecule would be capable of effector functions associated with the Fc domain when used in passive immunotherapy. These effector functions include antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cellular cytotoxicity (CDCC) which
20 promote the death of the cell to which the immunoglobulin molecule is specifically bound. The effector functions may therefore be desirable in therapeutic applications. Diagnostic assays include the ability to detect the presence of the immunoglobulin molecule. These assays
25 rely on the cross-linking of red cells or beads in agglutinations, the activation of complement in plaque assays, or the antigenic properties of the Fc region of the heavy chain as detected by secondary antibodies in ELISA or RIA procedures to detect the presence of the
30 immunoglobulin molecule. Such diagnostic assays can only be performed with the entire immunoglobulin molecule. The isolation of the immunoglobulin molecule is also facilitated by the presence of the Fc domain in that commonly used methods of immunoglobulin purification are
35 based upon interaction of reagents with the Fc domain. The preparation of a Fab fragment with the Fc domain is generally known in the immunological arts and can be accomplished by a variety of methods. A preferred method

of producing a Fab fragment with the Fc domain is described herein.

Particularly preferred is the immunoglobulin IgG1 human antibody described herein that is comprised of the b12 antibody Fab fragment and human Fc domain derived from an IgG1 subtype, designated b12 IgG1. The structure and preparation of this preferred human monoclonal antibody is described herein, and is prepared using the recombinant DNA expression vector pEE12. The complete nucleotide sequence of the vector for expression the complete heavy and light chains in the form of b12 IgG1 is shown in Figure 27 and also in SEQ ID NOs 156 and 170. Accordingly, the amino acid residue and nucleotide sequences, respectively, for a preferred complete heavy chain are shown in SEQ ID NOs 155 and 154, respectively, and for a preferred light chain are shown in SEQ ID NOs 153, and 152, respectively. The nucleotide sequences for preferred heavy and light chains are also shown in SEQ ID NOs 169 and 168, respectively.

C. Immunotherapeutic Methods and Compositions

The human monoclonal antibodies can also be used immunotherapeutically for HIV disease. The term "immunotherapeutically" or "immunotherapy" as used herein in conjunction with the monoclonal antibodies of the invention denotes both prophylactic as well as therapeutic administration. Thus, the monoclonal antibodies can be administered to high-risk patients in order to lessen the likelihood and/or severity of HIV-induced disease, administered to patients already evidencing active HIV infection, or administered to patients at risk of HIV infection.

1. Therapeutic Compositions

The present invention therefore contemplates therapeutic compositions useful for practicing the therapeutic methods described herein. Therapeutic compositions of the present invention contain

a physiologically tolerable carrier together with at least one species of human monoclonal antibody as described herein, dissolved or dispersed therein as an active ingredient. In a preferred embodiment, the therapeutic composition is not immunogenic when administered to a human patient for therapeutic purposes, unless that purpose is to induce an immune response, as described elsewhere herein.

As used herein, the terms "pharmaceutically acceptable", "physiologically tolerable" and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably and represent that the materials are capable of administration to or upon a human without the production of undesirable physiological effects such as nausea, dizziness, gastric upset and the like.

The preparation of a pharmacological composition that contains active ingredients dissolved or dispersed therein is well understood in the art. Typically such compositions are prepared as sterile injectables either as liquid solutions or suspensions, aqueous or non-aqueous, however, solid forms suitable for solution, or suspensions, in liquid prior to use can also be prepared. The preparation can also be emulsified.

The active ingredient can be mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient and in amounts suitable for use in the therapeutic methods described herein. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like and combinations thereof. In addition, if desired, the composition can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like which enhance the effectiveness of the active ingredient.

The therapeutic composition of the present invention can include pharmaceutically acceptable salts of the components therein. Pharmaceutically acceptable salts include the acid addition salts (formed with the free

amino groups of the polypeptide) that are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, tartaric, mandelic and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine and the like.

Physiologically tolerable carriers are well known in the art. Exemplary of liquid carriers are sterile aqueous solutions that contain no materials in addition to the active ingredients and water, or contain a buffer such as sodium phosphate at physiological pH value, physiological saline or both, such as phosphate-buffered saline. Still further, aqueous carriers can contain more than one buffer salt, as well as salts such as sodium and potassium chlorides, dextrose, propylene glycol, polyethylene glycol and other solutes.

Liquid compositions can also contain liquid phases in addition to and to the exclusion of water. Exemplary of such additional liquid phases are glycerin, vegetable oils such as cottonseed oil, organic esters such as ethyl oleate, and water-oil emulsions.

A therapeutic composition contains an HIV-neutralizing of a human monoclonal antibody of the present invention, typically an amount of at least 0.1 weight percent of antibody per weight of total therapeutic composition. A weight percent is a ratio by weight of antibody to total composition. Thus, for example, 0.1 weight percent is 0.1 grams of antibody per 100 grams of total composition.

2. Therapeutic Methods

In view of the demonstrated HIV neutralizing ability of the human monoclonal antibodies of the present invention, the present disclosure provides for a method for neutralizing HIV in vitro or in vivo. The

method comprises contacting a sample believed to contain HIV with a composition comprising a therapeutically effective amount of a human monoclonal antibody of this invention.

5 For in vivo modalities, the method comprises administering to the patient a therapeutically effective amount of a physiologically tolerable composition containing a human monoclonal antibody of the invention. Thus, the present invention describes in one embodiment a
10 method for providing passive immunotherapy to HIV disease in a human comprising administering to the human an immunotherapeutically effective amount of the monoclonal antibody of this invention.

 A representative patient for practicing the
15 present passive immunotherapeutic methods is any human exhibiting symptoms of HIV-induced disease, including AIDS or related conditions believed to be caused by HIV infection, and humans at risk of HIV infection. Patients at risk of infection by HIV include babies of HIV-infected
20 pregnant mothers, recipients of transfusions known to contain HIV, users of HIV contaminated needles, individuals who have participated in high risk sexual activities with known HIV-infected individuals, and the like risk situations.

25 In one embodiment, the passive immunization method comprises administering a composition comprising more than one species of human monoclonal antibody of this invention, preferably directed to non-competing epitopes or directed to distinct serotypes or strains of HIV, as to
30 afford increased effectiveness of the passive immunotherapy.

 A therapeutically (immunotherapeutically) effective amount of a human monoclonal antibody is a predetermined amount calculated to achieve the desired
35 effect, i.e., to neutralize the HIV present in the sample or in the patient, and thereby decrease the amount of detectable HIV in the sample or patient. In the case of in vivo therapies, an effective amount can be measured by

improvements in one or more symptoms associated with HIV-induced disease occurring in the patient, or by serological decreases in HIV antigens.

Thus, the dosage ranges for the administration of the monoclonal antibodies of the invention are those large enough to produce the desired effect in which the symptoms of the HIV disease are ameliorated or the likelihood of infection decreased. The dosage should not be so large as to cause adverse side effects, such as hyperviscosity syndromes, pulmonary edema, congestive heart failure, and the like. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient and can be determined by one of skill in the art.

The dosage can be adjusted by the individual physician in the event of any complication.

A therapeutically effective amount of an antibody of this invention is typically an amount of antibody such that when administered in a physiologically tolerable composition is sufficient to achieve a plasma concentration of from about 0.1 microgram (ug) per milliliter (ml) to about 100 ug/ml, preferably from about 1 ug/ml to about 5 ug/ml, and usually about 5 ug/ml. Stated differently, the dosage can vary from about 0.1 mg/kg to about 300 mg/kg, preferably from about 0.2 mg/kg to about 200 mg/kg, most preferably from about 0.5 mg/kg to about 20 mg/kg, in one or more dose administrations daily, for one or several days.

The human monoclonal antibodies of the invention can be administered parenterally by injection or by gradual infusion over time. Although the HIV infection is typically systemic and therefore most often treated by intravenous administration of therapeutic compositions, other tissues and delivery means are contemplated where there is a likelihood that the tissue targeted contains infectious HIV. Thus, human monoclonal antibodies of the invention can be administered intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity, transdermally, and can be delivered by

peristaltic means.

5 The therapeutic compositions containing a human monoclonal antibody of this invention are conventionally administered intravenously, as by injection of a unit dose, for example. The term "unit dose" when used in reference to a therapeutic composition of the present invention refers to physically discrete units suitable as unitary dosage for the subject, each unit containing a predetermined quantity of active material calculated to
10 produce the desired therapeutic effect in association with the required diluent; i.e., carrier, or vehicle.

The compositions are administered in a manner compatible with the dosage formulation, and in a therapeutically effective amount. The quantity to be
15 administered depends on the subject to be treated, capacity of the subject's system to utilize the active ingredient, and degree of therapeutic effect desired. Precise amounts of active ingredient required to be administered depend on the judgement of the practitioner and are peculiar to each individual. However, suitable dosage ranges for systemic application are disclosed herein and depend on the route of administration. Suitable regimes for administration are also variable, but are typified by an initial administration followed by
20 repeated doses at one or more hour intervals by a subsequent injection or other administration. Alternatively, continuous intravenous infusion sufficient to maintain concentrations in the blood in the ranges specified for in vivo therapies are contemplated.

30 As an aid to the administration of effective amounts of a monoclonal antibody, a diagnostic method for detecting a monoclonal antibody in the subject's blood is useful to characterize the fate of the administered therapeutic composition.

35 The invention also relates to a method for preparing a medicament or pharmaceutical composition comprising the human monoclonal antibodies of the invention, the medicament being used for immunotherapy of

HIV disease.

D. Diagnostic Assay Methods

5 The present invention contemplates various
assay methods for determining the presence, and preferably
amount, of HIV in a sample such as a biological fluid or
tissue sample using a human monoclonal antibody of this
invention as an immunochemical reagent to form an
immunoreaction product whose amount relates, either
10 directly or indirectly, to the amount of HIV in the
sample.

In a related embodiment, the present invention
contemplates various assay methods for determining the
presence, and preferably amount, of an anti-HIV antibody
15 present in a sample such as a biological fluid or tissue
sample from a HIV-infected individual using a human
monoclonal antibody of this invention as an immunochemical
reagent to form an immunoreaction product whose amount
relates, either directly or indirectly, to the amount of
20 anti-HIV antibody in the sample.

Those skilled in the art will understand that
there are numerous well known clinical diagnostic
chemistry procedures in which an immunochemical reagent of
this invention can be used to form an immunoreaction
25 product whose amount relates to the amount of HIV or anti-
HIV antibody present in a body sample. Thus, while
exemplary assay methods are described herein, the
invention is not so limited.

Various heterogenous and homogeneous protocols,
30 either competitive or noncompetitive, can be employed in
performing an assay method of this invention. Examples of
types of immunoassays which can utilize monoclonal
antibodies of the invention are competitive and non-
competitive immunoassays in either a direct or indirect
35 format. Examples of such immunoassays are the radioimmu-
noassay (RIA) and the sandwich (immunometric) assay.

Detection of the antigens using the monoclonal
antibodies of the invention can be done utilizing immun-

oassays which are run in either the forward, reverse, or simultaneous modes, including immunohistochemical assays on physiological samples. Those of skill in the art will know, or can readily discern, other immunoassay formats without undue experimentation.

The monoclonal antibodies of the invention can be bound to many different carriers and used to detect the presence of HIV. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding monoclonal antibodies, or will be able to ascertain such, using routine experimentation.

There are many different labels and methods of labeling known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, and bio-luminescent compounds. Those of ordinary skill in the art will know of other suitable labels for binding to the monoclonal antibodies of the invention, or will be able to ascertain such, using routine experimentation. Furthermore, the binding of these labels to the monoclonal antibodies of the invention can be done using standard techniques common to those of ordinary skill in the art.

For purposes of the invention, HIV may be detected by the monoclonal antibodies of the invention when present in samples of biological fluids and tissues. Any sample containing a detectable amount of HIV can be used. A sample can be a liquid such as urine, saliva, cerebrospinal fluid, blood, serum and the like, or a solid or semi-solid such as tissues, feces, and the like, or, alternatively, a solid tissue such as those commonly used in histological diagnosis.

Another labeling technique which may result in greater sensitivity consists of coupling the antibodies to low molecular weight haptens. These haptens can then be specifically detected by means of a second reaction. For example, it is common to use haptens such as biotin, which reacts with avidin, or dinitrophenol, pyridoxal, or fluorescein, which can react with specific anti-hapten antibodies.

The monoclonal antibodies of the invention are suited for use in vitro, for example, in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier for the detection of HIV in samples, as described above. The monoclonal antibodies in these immunoassays can be detectably labeled in various ways for in vitro use.

In using the human monoclonal antibodies of the invention for the in vivo detection of antigen, the detectably labeled human monoclonal antibody is given in a dose which is diagnostically effective. The term "diagnostically effective" means that the amount of detectably labeled human monoclonal antibody is administered in sufficient quantity to enable detection of the site having the HIV antigen for which the monoclonal antibodies are specific.

The concentration of detectably labeled human monoclonal antibody which is administered should be sufficient such that the binding to HIV is detectable compared to the background. Further, it is desirable that the detectably labeled monoclonal antibody be rapidly cleared from the circulatory system in order to give the best target-to-background signal ratio.

As a rule, the dosage of detectably labeled human monoclonal antibody for in vivo diagnosis will vary depending on such factors as age, sex, and extent of disease of the individual. The dosage of human monoclonal antibody can vary from about 0.01 mg/m² to about 500 mg/m², preferably 0.1 mg/m² to about 200 mg/m², most preferably about 0.1 mg/m² to about 10 mg/m². Such dosages may vary,

for example, depending on whether multiple injections are given, tissue, and other factors known to those of skill in the art.

5 For in vivo diagnostic imaging, the type of detection instrument available is a major factor in selecting a given radioisotope. The radioisotope chosen must have a type of decay which is detectable for a given type of instrument. Still another important factor in
10 selecting a radioisotope for in vivo diagnosis is that the half-life of the radioisotope be long enough so that it is still detectable at the time of maximum uptake by the target, but short enough so that deleterious radiation with respect to the host is minimized. Ideally, a radioisotope used for in vivo imaging will lack a particle
15 emission, but produce a large number of photons in the 140-250 keV range, which may be readily detected by conventional gamma cameras.

For in vivo diagnosis radioisotopes may be bound to immunoglobulin either directly or indirectly by using
20 an intermediate functional group. Intermediate functional groups which often are used to bind radioisotopes which exist as metallic ions to immunoglobulins are the bi-functional chelating agents such as diethylenetriam-
25 inepentacetic acid (DTPA) and ethylenediaminetetraacetic acid (EDTA) and similar molecules. Typical examples of metallic ions which can be bound to the monoclonal antibodies of the invention are ^{111}In , ^{97}Ru , ^{67}Ga , ^{68}Ga , ^{72}As , ^{89}Zr , and ^{201}Tl .

The monoclonal antibodies of the invention can
30 also be labeled with a paramagnetic isotope for purposes of in vivo diagnosis, as in magnetic resonance imaging (MRI) or electron spin resonance (ESR). In general, any conventional method for visualizing diagnostic imaging can be utilized. Usually gamma and positron emitting
35 radioisotopes are used for camera imaging and paramagnetic isotopes for MRI. Elements which are particularly useful in such techniques include ^{157}Gd , ^{55}Mn , ^{162}Dy , ^{52}Cr , and ^{56}Fe .

The human monoclonal antibodies of the invention can be used in vitro and in vivo to monitor the course of HIV disease therapy. Thus, for example, by measuring the increase or decrease in the number of cells infected with HIV or changes in the concentration of HIV present in the body or in various body fluids, it would be possible to determine whether a particular therapeutic regimen aimed at ameliorating the HIV disease is effective.

In a related diagnostic embodiment, the invention contemplates screening HIV-infected patients for the presence of circulating anti-HIV antibodies immunoreactive with gp120 that have a similar epitope immunospecificity when compared to a neutralizing antibody of this invention. Such a screening method indicates that the HIV-infected patient is exhibiting a significant immune response to the virus, and provides useful information regarding disease status and prognosis. The presence of anti-HIV antibodies cross-reactive with a neutralizing antibody of this invention indicates that the patient has some degree of HIV neutralizing activity, as defined herein.

The diagnostic assay involves determining whether the patient contains human anti-HIV antibodies immunoreactive with the same, similar or overlapping epitopes as a neutralizing antibody of the invention, such that there is a likelihood that there is a useful neutralizing immune response in the patient. There are a variety of immunological assay formats that can be utilized to determine cross-reactivity of test and control antibodies, and the invention need not be so limiting. Particularly preferred are competition assays for a common antigen, preferably in the solid phase.

A preferred embodiment of the competition immunoassay method comprises the steps of:

(1) contacting a sample believed to contain a human anti-HIV antibody with a diagnostically effective amount of the monoclonal antibody described herein that

binds mature gp120 in a competition immunoreaction admixture containing mature gp120 in the solid phase;

5 (2) maintaining said competition immunoreaction admixture under conditions sufficient for said monoclonal antibody to bind with said gp120 in the solid phase and form a solid phase immunoreactant; and

(3) detecting the amount of said immunoreactant present in said solid phase, and thereby the immunocompetence of any human anti-HIV antibody in
10 said sample.

A diagnostically effective amount, in this context, is a amount relative to the solid phase gp120, preferably "mature" gp120 as defined herein, sufficient to
15 produce a detectable solid phase immunoreaction product between the solid phase gp120 and the control anti-gp120 antibody of this invention. Exemplary competition assays are described herein using the preferred b12 antibody.

Conditions for conducting the competition immunoreaction are well known in the art and can be varied
20 according to recognized parameters in the contacting, the reaction admixtures, the maintenance step, the immunoreaction conditions and the detecting step. For example, the detection step can be conducted by use of a labeled antibody of this invention, by use of a second,
25 labeled anti-human antibody, and the like, as described herein.

E. Diagnostic Systems

The present invention also describes a
30 diagnostic system, preferably in kit form, for assaying for the presence of HIV or an anti-HIV antibody in a sample according to the diagnostic methods described herein. A diagnostic system includes, in an amount sufficient to perform at least one assay, a subject human
35 monoclonal antibody, as a separately packaged reagent.

In another embodiment, a diagnostic system is contemplated for assaying for the presence of an anti-HIV monoclonal antibody in a body fluid sample such as for

monitoring the fate of therapeutically administered antibody. The system includes, in an amount sufficient for at least one assay, a subject antibody as a control reagent, and preferably a preselected amount of HIV antigen, each as separately packaged immunochemical reagents.

Instructions for use of the packaged reagent are also typically included.

"Instructions for use" typically include a tangible expression describing the reagent concentration or at least one assay method parameter such as the relative amounts of reagent and sample to be admixed, maintenance time periods for reagent/ sample admixtures, temperature, buffer conditions and the like.

In embodiments for detecting HIV or anti-HIV antibody in a body fluid, a diagnostic system of the present invention can include a label or indicating means capable of signaling the formation of an immunocomplex containing a human monoclonal antibody of the present invention.

The word "complex" as used herein refers to the product of a specific binding reaction such as an antibody-antigen reaction. Exemplary complexes are immunoreaction products.

As used herein, the terms "label" and "indicating means" in their various grammatical forms refer to single atoms and molecules that are either directly or indirectly involved in the production of a detectable signal to indicate the presence of a complex. Any label or indicating means can be linked to or incorporated in an expressed protein, polypeptide, or antibody molecule that is part of an antibody or monoclonal antibody composition of the present invention, or used separately, and those atoms or molecules can be used alone or in conjunction with additional reagents. Such labels are themselves well-known in clinical diagnostic chemistry and constitute a part of this invention only insofar as they are utilized with otherwise novel proteins methods and/or systems.

The labeling means can be a fluorescent labeling agent that chemically binds to antibodies or antigens without denaturing them to form a fluorochrome (dye) that is a useful immunofluorescent tracer. Suitable fluorescent labeling agents are fluorochromes such as fluorescein isocyanate (FIC), fluorescein isothiocyanate (FITC), 5-dimethylamine-1-naphthalenesulfonyl chloride (DANSC), tetramethylrhodamine isothiocyanate (TRITC), lissamine, rhodamine 8200 sulphonyl chloride (RB 200 SC) and the like. A description of immunofluorescence analysis techniques is found in DeLuca, "Immunofluorescence Analysis", in Antibody As a Tool, Marchalonis et al., eds., John Wiley & Sons, Ltd., pp. 189-231 (1982), which is incorporated herein by reference.

In preferred embodiments, the indicating group is an enzyme, such as horseradish peroxidase (HRP), glucose oxidase, or the like. In such cases where the principal indicating group is an enzyme such as HRP or glucose oxidase, additional reagents are required to visualize the fact that a receptor-ligand complex (immunoreactant) has formed. Such additional reagents for HRP include hydrogen peroxide and an oxidation dye precursor such as diaminobenzidine. An additional reagent useful with glucose oxidase is 2,2'-amino-di-(3-ethyl-benzthiazoline-G-sulfonic acid) (ABTS).

Radioactive elements are also useful labeling agents and are used illustratively herein. An exemplary radiolabeling agent is a radioactive element that produces gamma ray emissions. Elements which themselves emit gamma rays, such as ^{124}I , ^{125}I , ^{128}I , ^{132}I and ^{51}Cr represent one class of gamma ray emission-producing radioactive element indicating groups. Particularly preferred is ^{125}I . Another group of useful labeling means are those elements such as ^{11}C , ^{18}F , ^{15}O and ^{13}N which themselves emit positrons. The positrons so emitted produce gamma rays upon encounters with electrons present in the animal's body. Also useful is a beta emitter, such ^{111}In indium of ^3H .

5 The linking of labels, i.e., labeling of, polypeptides and proteins is well known in the art. For instance, antibody molecules produced by a hybridoma can be labeled by metabolic incorporation of radioisotope-containing amino acids provided as a component in the culture medium. See, for example, Galfre et al., Meth. Enzymol., 73:3-46 (1981). The techniques of protein conjugation or coupling through activated functional groups are particularly applicable. See, for example, 10 Aurameas et al., Scand. J. Immunol., Vol. 8 Suppl. 7:7-23 (1978), Rodwell et al., Biotech., 3:889-894 (1984), and U.S. Pat. No. 4,493,795.

 The diagnostic systems can also include, preferably as a separate package, a specific binding 15 agent. A "specific binding agent" is a molecular entity capable of selectively binding a reagent species of the present invention or a complex containing such a species, but is not itself a polypeptide or antibody molecule composition of the present invention. Exemplary specific 20 binding agents are second antibody molecules, complement proteins or fragments thereof, S. aureus protein A, and the like. Preferably the specific binding agent binds the reagent species when that species is present as part of a complex.

25 In preferred embodiments, the specific binding agent is labeled. However, when the diagnostic system includes a specific binding agent that is not labeled, the agent is typically used as an amplifying means or reagent. In these embodiments, the labeled specific binding agent 30 is capable of specifically binding the amplifying means when the amplifying means is bound to a reagent species-containing complex.

 The diagnostic kits of the present invention can be used in an "ELISA" format to detect the quantity of an 35 antigen or antibody of this invention in a vascular fluid sample such as blood, serum, or plasma. "ELISA" refers to an enzyme-linked immunosorbent assay that employs an antibody or antigen bound to a solid phase and an enzyme-

antigen or enzyme-antibody conjugate to detect and quantify the amount of an antigen present in a sample. A description of the ELISA technique is found in Chapter 22 of the 4th Edition of Basic and Clinical Immunology by D.P. Sites et al., published by Lange Medical Publications of Los Altos, CA in 1982 and in U.S. Patents No. 3,654,090; No. 3,850,752; and No. 4,016,043, which are all incorporated herein by reference.

Thus, in some embodiments, a human monoclonal antibody of the present invention can be affixed to a solid matrix to form a solid support that comprises a package in the subject diagnostic systems.

A reagent is typically affixed to a solid matrix by adsorption from an aqueous medium although other modes of affixation applicable to proteins and polypeptides well known to those skilled in the art, can be used.

Useful solid matrices are also well known in the art. Such materials are water insoluble and include the cross-linked dextran available under the trademark SEPHADEX from Pharmacia Fine Chemicals (Piscataway, NJ); agarose; beads of polystyrene beads about 1 micron to about 5 millimeters in diameter available from Abbott Laboratories of North Chicago, IL; polyvinyl chloride, polystyrene, cross-linked polyacrylamide, nitrocellulose or nylon-based webs such as sheets, strips or paddles; or tubes, plates or the wells of a microtiter plate such as those made from polystyrene or polyvinylchloride.

The reagent species, labeled specific binding agent or amplifying reagent of any diagnostic system described herein can be provided in solution, as a liquid dispersion or as a substantially dry power, e.g., in lyophilized form. Where the indicating means is an enzyme, the enzyme's substrate can also be provided in a separate package of a system. A solid support such as the before-described microtiter plate and one or more buffers can also be included as separately packaged elements in this diagnostic assay system.

The packaging materials discussed herein in relation to diagnostic systems are those customarily utilized in diagnostic systems.

5 The term "package" refers to a solid matrix or material such as glass, plastic (e.g., polyethylene, polypropylene and polycarbonate), paper, foil and the like capable of holding within fixed limits a diagnostic reagent such as a monoclonal antibody of the present invention. Thus, for example, a package can be a bottle, 10 vial, plastic and plastic-foil laminated envelope or the like container used to contain a contemplated diagnostic reagent or it can be a microtiter plate well to which microgram quantities of a contemplated diagnostic reagent have been operatively affixed, i.e., linked so as to be 15 capable of being immunologically bound by an antibody or polypeptide to be detected.

 The materials for use in the assay of the invention are ideally suited for the preparation of a kit. Such a kit may comprise a carrier means being 20 compartmentalized to receive in close confinement one or more container means such as vials, tubes, and the like, each of the container means comprising one of the separate elements to be used in the method. For example, one of the container means may comprise a human monoclonal 25 antibody of the invention which is, or can be, detectably labelled. The kit may also have containers containing any of the other above-recited immunochemical reagents used to practice the diagnostic methods.

30 F. Methods for Producing an HIV-Neutralizing Human Monoclonal Antibody

 The present invention describes methods for producing novel HIV-neutralizing human monoclonal antibodies. The methods are based generally on the use of 35 combinatorial libraries of antibody molecules which can be produced from a variety of sources, and include naive libraries, modified libraries, and libraries produced directly from human donors exhibiting an HIV-specific

immune response.

5 The combinatorial library production and manipulation methods have been extensively described in the literature, and will not be reviewed in detail herein, except for those feature required to make and use unique
10 embodiments of the present invention. However, the methods generally involve the use of a filamentous phage (phagemid) surface expression vector system for cloning and expressing antibody species of the library. Various phagemid cloning systems to produce combinatorial
15 libraries have been described by others. See, for example the preparation of combinatorial antibody libraries on phagemids as described by Kang et al., Proc. Natl. Acad. Sci., USA, 88:4363-4366 (1991); Barbas et al., Proc. Natl. Acad. Sci., USA, 88:7978-7982 (1991); Zebedee et al., Proc. Natl. Acad. Sci., USA, 89:3175-3179 (1992); Kang et al., Proc. Natl. Acad. Sci., USA, 88:11120-11123 (1991); Barbas et al., Proc. Natl. Acad. Sci., USA, 89:4457-4461 (1992); and Gram et al., Proc. Natl. Acad. Sci., USA, 89:3576-3580 (1992), which references are hereby
20 incorporated by reference.

In one embodiment, the method involves preparing a phagemid library of human monoclonal antibodies by using donor immune cell messenger RNA from HIV-infected donors.
25 The donors can be symptomatic of AIDS, but in preferred embodiments the donor is asymptomatic, as the resulting library contains a substantially higher number of HIV-neutralizing human monoclonal antibodies.

In another embodiment, the donor is naive relative to an immune response to HIV, i.e., the donor is not HIV-infected. Alternatively, the library can be synthetic, or
30 can be derived from a donor who has an immune response to other antigens.

The method for producing a human monoclonal antibody generally involves (1) preparing separate H and L
35 chain-encoding gene libraries in cloning vectors using human immunoglobulin genes as a source for the libraries, (2) combining the H and L chain encoding gene libraries

into a single dicistronic expression vector capable of
expressing and assembling a heterodimeric antibody
molecule, (3) expressing the assembled heterodimeric
antibody molecule on the surface of a filamentous phage
5 particle, (4) isolating the surface-expressed phage
particle using immunoaffinity techniques such as panning
of phage particles against a preselected antigen, thereby
isolating one or more species of phagemid containing
particular H and L chain-encoding genes and antibody
10 molecules that immunoreact with the preselected antigen.

As described herein the Examples, the resulting
phagemid library can be manipulated to increase and/or
alter the immunospecificities of the monoclonal antibodies
of the library to produce and subsequently identify
15 additional, desirable, human monoclonal antibodies of the
present invention.

For example, the heavy (H) chain and light (L)
chain immunoglobulin molecule encoding genes can be
randomly mixed (shuffled) to create new HL pairs in an
20 assembled immunoglobulin molecule. Additionally, either
or both the H and L chain encoding genes can be
mutagenized in the complementarity determining region
(CDR) of the variable region of the immunoglobulin
polypeptide, and subsequently screened for desirable
25 immunoreaction and neutralization capabilities.

In one embodiment, the H and L genes can be cloned
into separate, monocistronic expression vectors, referred
to as a "binary" system described further herein. In this
method, step (2) above differs in that the combining of H
30 and L chain encoding genes occurs by the co-introduction
of the two binary plasmids into a single host cell for
expression and assembly of a phagemid having the surface
accessible antibody heterodimer molecule.

In one shuffling embodiment, the shuffling can be
35 accomplished with the binary expression vectors, each
capable of expressing a single heavy or light chain
encoding gene, as described in Example 11.

In the present methods, the antibody molecules are monoclonal because the cloning methods allow for the preparation of clonally pure species of antibody producing cell lines. In addition, the monoclonal antibodies are human because the H and L chain encoding genes are derived from human immunoglobulin producing immune cells, such as spleen, thymus, bone marrow, and the like.

The method of producing a HIV-neutralizing human monoclonal antibody also requires that the resulting antibody library, immunoreactive with a preselected HIV antigen, is screened for the presence of antibody species which have the capacity to neutralize HIV in one or more of the assays described herein for determining neutralization capacity. Thus, a preferred library of antibody molecules is first produced which binds to an HIV antigen, preferably gp160, gp120, gp41, the V3 loop region of gp160, or the CD4 binding site of gp120 and gp41, and then is screened for the presence of HIV-neutralizing antibodies as described herein.

Additional libraries can be screened from shuffled libraries for additional HIV-immunoreactive and neutralizing human monoclonal antibodies.

As a further characterization of the present invention the nucleotide and corresponding amino acid residue sequence of the antibody molecule's H or L chain encoding gene is determined by nucleic acid sequencing. The primary amino acid residue sequence information provides essential information regarding the antibody molecule's epitope reactivity.

Sequence comparisons of identified HIV-immunoreactive monoclonal antibody variable chain region sequences are shown herein in Figures 10-13. The sequences are aligned based on sequence homology, and groups of related antibody molecules are identified thereby in which heavy chain or light chain genes share substantial sequence homology.

An exemplary preparation of a human monoclonal antibody is described in the Examples. The isolation of a

particular vector capable of expressing an antibody of interest involves the introduction of the dicistronic expression vector into a host cell permissive for expression of filamentous phage genes and the assembly of phage particles. Where the binary vector system is used, both vectors are introduced in the host cell. Typically, the host is E. coli. Thereafter, a helper phage genome is introduced into the host cell containing the immunoglobulin expression vector(s) to provide the genetic complementation necessary to allow phage particles to be assembled. The resulting host cell is cultured to allow the introduced phage genes and immunoglobulin genes to be expressed, and for phage particles to be assembled and shed from the host cell. The shed phage particles are then harvested (collected) from the host cell culture media and screened for desirable immunoreaction and neutralization properties. Typically, the harvested particles are "panned" for immunoreaction with a preselected antigen. The strongly immunoreactive particles are then collected, and individual species of particles are clonally isolated and further screened for HIV neutralization. Phage which produce neutralizing antibodies are selected and used as a source of a human HIV neutralizing monoclonal antibody of this invention.

Human monoclonal antibodies of this invention can also be produced by altering the nucleotide sequence of a polynucleotide sequence that encodes a heavy or light chain of a monoclonal antibody of this invention. For example, by site directed mutagenesis, one can alter the nucleotide sequence of an expression vector and thereby introduce changes in the resulting expressed amino acid residue sequence. Thus one can take the polynucleotide of SEQ ID NO 66, for example, and convert it into the polynucleotide of SEQ ID NO 67. Similarly, one can take a known polynucleotide and randomly alter it by random mutagenesis, reintroduce the altered polynucleotide into an expression system and subsequently screen the product H:L pair for HIV-neutralizing activity.

Site-directed and random mutagenesis methods are well known in the polynucleotide arts, and are not to be construed as limiting as methods for altering the nucleotide sequence of a subject polynucleotide.

5 Due to the presence of the phage particle in an immunoaffinity isolated antibody, one embodiment involves the manipulation of the resulting cloned genes to truncate the immunoglobulin-coding gene such that a soluble Fab
10 fragment is secreted by the host E. coli cell containing the phagemid vector. Thus, the resulting manipulated cloned immunoglobulin genes produce a soluble Fab which can be readily characterized in ELISA assays for epitope binding studies, in competition assays with known anti-HIV
15 antibody molecules, and in HIV neutralization assays. The solubilized Fab provides a reproducible and comparable antibody preparation for comparative and characterization studies.

The preparation of soluble Fab is generally described in the immunological arts, and can be conducted
20 as described herein in Example 2b6), or as described by Burton et al., Proc. Natl. Acad. Sci., USA, 88:10134-10137 (1991).

25 G. Expression Vectors and Polynucleotides for Expressing Anti-HIV Monoclonal Antibodies

The preparation of human monoclonal antibodies of this invention depends, in one embodiment, on the cloning and expression vectors used to prepare the combinatorial antibody libraries described herein. The
30 cloned immunoglobulin heavy and light chain genes can be shuttled between lambda vectors, phagemid vectors and plasmid vectors at various stages of the methods described herein.

35 The phagemid vectors produce fusion proteins that are expressed on the surface of an assembled filamentous phage particle.

A preferred phagemid vector of the present invention is a recombinant DNA (rDNA) molecule containing

a nucleotide sequence that codes for and is capable of expressing a fusion polypeptide containing, in the direction of amino- to carboxy-terminus, (1) a prokaryotic secretion signal domain, (2) a heterologous polypeptide defining an immunoglobulin heavy or light chain variable region, and (3) a filamentous phage membrane anchor domain. The vector includes DNA expression control sequences for expressing the fusion polypeptide, preferably prokaryotic control sequences.

The filamentous phage membrane anchor is preferably a domain of the cpIII or cpVIII coat protein capable of associating with the matrix of a filamentous phage particle, thereby incorporating the fusion polypeptide onto the phage surface.

The secretion signal is a leader peptide domain of a protein that targets the protein to the periplasmic membrane of gram negative bacteria. A preferred secretion signal is a pelB secretion signal. The predicted amino acid residue sequences of the secretion signal domain from two pelB gene product variants from *Erwinia carotova* are described in Lei et al., Nature, 331:543-546 (1988).

The leader sequence of the pelB protein has previously been used as a secretion signal for fusion proteins (Better et al., Science, 240:1041-1043 (1988); Sastry et al., Proc. Natl. Acad. Sci., USA, 86:5728-5732 (1989); and Mullinax et al., Proc. Natl. Acad. Sci., USA, 87:8095-8099 (1990)). Amino acid residue sequences for other secretion signal polypeptide domains from *E. coli* useful in this invention as described in Oliver, Escherichia coli and Salmonella Typhimurium, Neidhard, F.C. (ed.), American Society for Microbiology, Washington, D.C., 1:56-69 (1987).

Preferred membrane anchors for the vector are obtainable from filamentous phage M13, f1, fd, and equivalent filamentous phage. Preferred membrane anchor domains are found in the coat proteins encoded by gene III and gene VIII. The membrane anchor domain of a filamentous phage coat protein is a portion of the carboxy

terminal region of the coat protein and includes a region of hydrophobic amino acid residues for spanning a lipid bilayer membrane, and a region of charged amino acid residues normally found at the cytoplasmic face of the membrane and extending away from the membrane.

In the phage f1, gene VIII coat protein's membrane spanning region comprises residue Trp-26 through Lys-40, and the cytoplasmic region comprises the carboxy-terminal 11 residues from 41 to 52 (Ohkawa et al., J. Biol. Chem., 256:9951-9958 (1981)). An exemplary membrane anchor would consist of residues 26 to 40 of cpVIII. Thus, the amino acid residue sequence of a preferred membrane anchor domain is derived from the M13 filamentous phage gene VIII coat protein (also designated cpVIII or CP 8). Gene VIII coat protein is present on a mature filamentous phage over the majority of the phage particle with typically about 2500 to 3000 copies of the coat protein.

In addition, the amino acid residue sequence of another preferred membrane anchor domain is derived from the M13 filamentous phage gene III coat protein (also designated cpIII). Gene III coat protein is present on a mature filamentous phage at one end of the phage particle with typically about 4 to 6 copies of the coat protein.

For detailed descriptions of the structure of filamentous phage particles, their coat proteins and particle assembly, see the reviews by Rached et al., Microbiol. Rev., 50:401-427 (1986); and Model et al., in "The Bacteriophages: Vol. 2", R. Calendar, ed. Plenum Publishing Co., pp. 375-456 (1988).

DNA expression control sequences comprise a set of DNA expression signals for expressing a structural gene product and include both 5' and 3' elements, as is well known, operatively linked to the cistron such that the cistron is able to express a structural gene product. The 5' control sequences define a promoter for initiating transcription and a ribosome binding site operatively linked at the 5' terminus of the upstream translatable DNA sequence.

To achieve high levels of gene expression in E. coli, it is necessary to use not only strong promoters to generate large quantities of mRNA, but also ribosome binding sites to ensure that the mRNA is efficiently translated. In E. coli, the ribosome binding site includes an initiation codon (AUG) and a sequence 3-9 nucleotides long located 3-11 nucleotides upstream from the initiation codon (Shine et al., Nature, 254:34 (1975)). The sequence, AGGAGGU, which is called the Shine-Dalgarno (SD) sequence, is complementary to the 3' end of E. coli 16S rRNA. Binding of the ribosome to mRNA and the sequence at the 3' end of the mRNA can be affected by several factors:

- (i) The degree of complementarity between the SD sequence and 3' end of the 16S rRNA.
- (ii) The spacing and possibly the DNA sequence lying between the SD sequence and the AUG. Roberts et al., Proc. Natl. Acad. Sci. USA, 76:760, (1979a); Roberts et al., Proc. Natl. Acad. Sci. USA, 76:5596 (1979b); Guarente et al., Science, 209:1428 (1980); and Guarente et al., Cell, 20:543 (1980). Optimization is achieved by measuring the level of expression of genes in plasmids in which this spacing is systematically altered. Comparison of different mRNAs shows that there are statistically preferred sequences from positions -20 to +13 (where the A of the AUG is position 0). Gold et al., Annu. Rev. Microbiol., 35:365 (1981). Leader sequences have been shown to influence translation dramatically. Roberts et al., 1979 a, b supra.
- (iii) The nucleotide sequence following the AUG, which affects ribosome binding. Taniguchi et al., J. Mol. Biol., 118:533 (1978).

The 3' control sequences define at least one termination (stop) codon in frame with and operatively linked to the heterologous fusion polypeptide.

In preferred embodiments, the vector utilized includes a prokaryotic origin of replication or replicon, i.e., a DNA sequence having the ability to direct

autonomous replication and maintenance of the recombinant DNA molecule extra chromosomally in a prokaryotic host cell, such as a bacterial host cell, transformed therewith. Such origins of replication are well known in the art. Preferred origins of replication are those that are efficient in the host organism. A preferred host cell is E. coli. For use of a vector in E. coli, a preferred origin of replication is ColE1 found in pBR322 and a variety of other common plasmids. Also preferred is the p15A origin of replication found on pACYC and its derivatives. The ColE1 and p15A replicon have been extensively utilized in molecular biology, are available on a variety of plasmids and are described at least by Sambrook et al., in "Molecular Cloning: a Laboratory Manual", 2nd edition, Cold Spring Harbor Laboratory Press (1989).

The ColE1 and p15A replicons are particularly preferred for use in one embodiment of the present invention where two "binary" plasmids are utilized because they each have the ability to direct the replication of plasmid in E. coli while the other replicon is present in a second plasmid in the same E. coli cell. In other words, ColE1 and p15A are non-interfering replicons that allow the maintenance of two plasmids in the same host (see, for example, Sambrook et al., supra, at pages 1.3-1.4). This feature is particularly important in the binary vectors embodiment of the present invention because a single host cell permissive for phage replication must support the independent and simultaneous replication of two separate vectors, namely a first vector for expressing a heavy chain polypeptide, and a second vector for expressing a light chain polypeptide.

In addition, those embodiments that include a prokaryotic replicon can also include a gene whose expression confers a selective advantage, such as drug resistance, to a bacterial host transformed therewith. Typical bacterial drug resistance genes are those that confer resistance to ampicillin, tetracycline,

neomycin/kanamycin or cholamphenicol. Vectors typically also contain convenient restriction sites for insertion of translatable DNA sequences. Exemplary vectors are the plasmids pUC8, pUC9, pBR322, and pBR329 available from BioRad Laboratories, (Richmond, CA) and pPL and pKK223 available from Pharmacia, (Piscataway, NJ).

A vector for expression of a monoclonal antibody of the invention on the surface of a filamentous phage particle is a recombinant DNA (rDNA) molecule adapted for receiving and expressing translatable first and second DNA sequences in the form of first and second polypeptides wherein one of the polypeptides is fused to a filamentous phage coat protein membrane anchor. That is, at least one of the polypeptides is a fusion polypeptide containing a filamentous phage membrane anchor domain, a prokaryotic secretion signal domain, and an immunoglobulin heavy or light chain variable domain.

A DNA expression vector for expressing a heterodimeric antibody molecule provides a system for independently cloning (inserting) the two translatable DNA sequences into two separate cassettes present in the vector, to form two separate cistrons for expressing the first and second polypeptides of the antibody molecule, or the ligand binding portions of the polypeptides that comprise the antibody molecule (i.e., the H and L variable regions of an immunoglobulin molecule). The DNA expression vector for expressing two cistrons is referred to as a dicistronic expression vector.

The vector comprises a first cassette that includes upstream and downstream translatable DNA sequences operatively linked via a sequence of nucleotides adapted for directional ligation to an insert DNA. The upstream translatable sequence encodes the secretion signal as defined herein. The downstream translatable sequence encodes the filamentous phage membrane anchor as defined herein. The cassette preferably includes DNA expression control sequences for expressing the receptor polypeptide that is produced when an insert translatable

DNA sequence (insert DNA) is directionally inserted into the cassette via the sequence of nucleotides adapted for directional ligation. The filamentous phage membrane anchor is preferably a domain of the cpIII or cpVIII coat protein capable of binding the matrix of a filamentous phage particle, thereby incorporating the fusion polypeptide onto the phage surface.

The receptor expressing vector also contains a second cassette for expressing a second receptor polypeptide. The second cassette includes a second translatable DNA sequence that encodes a secretion signal, as defined herein, operatively linked at its 3' terminus via a sequence of nucleotides adapted for directional ligation to a downstream DNA sequence of the vector that typically defines at least one stop codon in the reading frame of the cassette. The second translatable DNA sequence is operatively linked at its 5' terminus to DNA expression control sequences forming the 5' elements. The second cassette is capable, upon insertion of a translatable DNA sequence (insert DNA), of expressing the second fusion polypeptide comprising a receptor of the secretion signal with a polypeptide coded by the insert DNA.

An upstream translatable DNA sequence encodes a prokaryotic secretion signal as described earlier. The upstream translatable DNA sequence encoding the pelB secretion signal is a preferred DNA sequence for inclusion in a receptor expression vector. A downstream translatable DNA sequence encodes a filamentous phage membrane anchor as described earlier. Thus, a downstream translatable DNA sequence encodes an amino acid residue sequence that corresponds, and preferably is identical, to the membrane anchor domain of either a filamentous phage gene III or gene VIII coat polypeptide.

A cassette in a DNA expression vector of this invention is the region of the vector that forms, upon insertion of a translatable DNA sequence (insert DNA), a sequence of nucleotides capable of expressing, in an

appropriate host, a fusion polypeptide. The expression-competent sequence of nucleotides is referred to as a cistron. Thus, the cassette comprises DNA expression control elements operatively linked to the upstream and downstream translatable DNA sequences. A cistron is formed when a translatable DNA sequence is directionally inserted (directionally ligated) between the upstream and downstream sequences via the sequence of nucleotides adapted for that purpose. The resulting three translatable DNA sequences, namely the upstream, the inserted and the downstream sequences, are all operatively linked in the same reading frame.

Thus, a DNA expression vector for expressing an antibody molecule provides a system for cloning translatable DNA sequences into the cassette portions of the vector to produce cistrons capable of expressing the first and second polypeptides, i.e., the heavy and light chains of a monoclonal antibody.

As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting between different genetic environments another nucleic acid to which it has been operatively linked. Preferred vectors are those capable of autonomous replication and expression of structural gene products present in the DNA segments to which they are operatively linked. Vectors, therefore, preferably contain the replicons and selectable markers described earlier.

As used herein with regard to DNA sequences or segments, the phrase "operatively linked" means the sequences or segments have been covalently joined, preferably by conventional phosphodiester bonds, into one strand of DNA, whether in single or double stranded form. The choice of vector to which transcription unit or a cassette of this invention is operatively linked depends directly, as is well known in the art, on the functional properties desired, e.g., vector replication and protein expression, and the host cell to be transformed, these being limitations inherent in the art of constructing

recombinant DNA molecules.

5 A sequence of nucleotides adapted for directional ligation, i.e., a polylinker, is a region of the DNA expression vector that (1) operatively links for replication and transport the upstream and downstream translatable DNA sequences and (2) provides a site or means for directional ligation of a DNA sequence into the vector. Typically, a directional polylinker is a sequence of nucleotides that defines two or more restriction endonuclease recognition sequences, or restriction sites. Upon restriction cleavage, the two sites yield cohesive termini to which a translatable DNA sequence can be ligated to the DNA expression vector. Preferably, the two restriction sites provide, upon restriction cleavage, 15 cohesive termini that are non-complementary and thereby permit directional insertion of a translatable DNA sequence into the cassette. In one embodiment, the directional ligation means is provided by nucleotides present in the upstream translatable DNA sequence, 20 downstream translatable DNA sequence, or both. In another embodiment, the sequence of nucleotides adapted for directional ligation comprises a sequence of nucleotides that defines multiple directional cloning means. Where the sequence of nucleotides adapted for directional ligation defines numerous restriction sites, it is 25 referred to as a multiple cloning site.

In a preferred embodiment, a DNA expression vector is designed for convenient manipulation in the form of a filamentous phage particle encapsulating a genome 30 according to the teachings of the present invention. In this embodiment, a DNA expression vector further contains a nucleotide sequence that defines a filamentous phage origin of replication such that the vector, upon presentation of the appropriate genetic complementation, 35 can replicate as a filamentous phage in single stranded replicative form and be packaged into filamentous phage particles. This feature provides the ability of the DNA expression vector to be packaged into phage particles for

subsequent segregation of the particle, and vector contained therein, away from other particles that comprise a population of phage particles.

5 A filamentous phage origin of replication is a region of the phage genome, as is well known, that defines sites for initiation of replication, termination of replication and packaging of the replicative form produced by replication (see, for example, Rasched et al., Microbiol. Rev., 50:401-427 (1986); and Horiuchi, J. Mol. Biol., 188:215-223 (1986)).

10 A preferred filamentous phage origin of replication for use in the present invention is an M13, f1 or fd phage origin of replication (Short et al., Nucl. Acids Res., 16:7583-7600 (1988)). Preferred DNA
15 expression vectors for cloning and expression a human monoclonal antibody of this invention are the dicistronic expression vectors pComb8, pComb2-8, pComb3, pComb2-3 and pComb2-3', described herein.

20 A particularly preferred vector of the present invention includes a polynucleotide sequence that encodes a heavy or light chain variable region of a human monoclonal antibody of the present invention. Particularly preferred are vectors that include a
25 nucleotide sequence that encodes a heavy or light chain amino acid residue sequence shown in Figures 10-13, that encodes a heavy or light chain having the binding specificity of those sequences shown in Figures 10-13, or that encodes a heavy or light chain having conservative substitutions relative to a sequence shown in Figures 10-
30 13, and complementary polynucleotide sequences thereto.

Insofar as polynucleotides are component parts of a DNA expression vector for producing a human monoclonal antibody heavy or light chain immunoglobulin variable region amino acid residue sequence, the invention also
35 contemplates isolated polynucleotides that encode such heavy or light chain sequences.

It is to be understood that, due to the genetic code and its attendant redundancies, numerous

polynucleotide sequences can be designed that encode a contemplated heavy or light chain immunoglobulin variable region amino acid residue sequence. Thus, the invention contemplates such alternate polynucleotide sequences incorporating the features of the redundancy of the genetic code.

Insofar as the expression vector for producing a human monoclonal antibody of this invention is carried in a host cell compatible with expression of the antibody, the invention contemplates a host cell containing a vector or polynucleotide of this invention. A preferred host cell is E. coli, as described herein.

E. coli cultures containing preferred expression vectors that produce a human monoclonal antibody of this invention were deposited pursuant to Budapest Treaty requirements with the American Type Culture Collection (ATCC), Rockville, MD, as described herein.

Examples

The following examples are intended to illustrate, but not limit, the scope of the invention.

1. Construction of a Dicistronic Expression Vector for Producing a Heterodimeric Receptor on Phage Particles

To obtain a vector system for generating a large number of Fab antibody fragments that can be screened directly, expression libraries in bacteriophage Lambda have previously been constructed as described in Huse et al., Science, 246:1275-1281 (1989). These systems did not contain design features that provide for the expressed Fab to be targeted to the surface of a filamentous phage particle.

The main criterion used in choosing a vector system was the necessity of generating the largest number of Fab fragments which could be screened directly. Bacteriophage Lambda was selected as the starting point to develop an expression vector for three reasons. First, in

vitro packaging of phage DNA was the most efficient method of reintroducing DNA into host cells. Second, it was possible to detect protein expression at the level of single phage plaques. Finally, the screening of phage libraries typically involved less difficulty with nonspecific binding. The alternative, plasmid cloning vectors, are only advantageous in the analysis of clones after they have been identified. This advantage was not lost in the present system because of the use of a dicistronic expression vector such as pCombVIII, thereby permitting a plasmid containing the heavy chain, light chain, or Fab expressing inserts to be excised.

a. Construction of Dicistronic Expression Vector pCOMB

1) Preparation of Lambda Zap™ II

Lambda Zap™ II is a derivative of the original Lambda Zap (ATCC Accession No. 40,298) that maintains all of the characteristics of the original Lambda Zap including 6 unique cloning sites, fusion protein expression, and the ability to rapidly excise the insert in the form of a phagemid (Bluescript SK-), but lacks the SAM 100 mutation, allowing growth on many Non-Sup F strains, including XL1-Blue. The Lambda Zap™ II was constructed as described in Short et al., Nuc. Acids Res., 16:7583-7600, 1988, by replacing the Lambda S gene contained in a 4254 base pair (bp) DNA fragment produced by digesting Lambda Zap with the restriction enzyme Nco I. This 4254 bp DNA fragment was replaced with the 4254 bp DNA fragment containing the Lambda S gene isolated from Lambda gt10 (ATCC # 40,179) after digesting the vector with the restriction enzyme Nco I. The 4254 bp DNA fragment isolated from lambda gt10 was ligated into the original Lambda Zap vector using T4 DNA ligase and standard protocols such as those described in Current Protocols in Molecular Biology, Ausubel et al., eds., John Wiley and Sons, NY, 1987, to form Lambda Zap™ II.

2) Preparation of Lambda Hc2

To express a plurality of V_H-coding DNA homologs in an E. coli host cell, a vector designated Lambda Hc2 was constructed. The vector provided the following: the capacity to place the V_H-coding DNA homologs in the proper reading frame; a ribosome binding site as described by Shine et al., Nature, 254:34 (1975); a leader sequence directing the expressed protein to the periplasmic space designated the pelB secretion signal; a polynucleotide sequence that coded for a known epitope (epitope tag); and also a polynucleotide that coded for a spacer protein between the V_H-coding DNA homolog and the polynucleotide coding for the epitope tag. Lambda Hc2 has been previously described by Huse et al., Science, 246:1275-1281 (1989).

To prepare Lambda Hc2, a synthetic DNA sequence containing all of the above features was constructed by designing single stranded polynucleotide segments of 20-40 bases that would hybridize to each other and form the double stranded synthetic DNA sequence shown in Figure 1. The individual single-stranded polynucleotide segments are shown in Table 1.

Polynucleotides N2, N3, N9-4, N11, N10-5, N6, N7 and N8 (Table 1) were kinased by adding 1 μ l of each polynucleotide 0.1 micrograms/microliter (μ g/ μ l) and 20 units of T₄ polynucleotide kinase to a solution containing 70 mM Tris-HCl (Tris[hydroxymethyl] aminomethane hydrochloride) at pH 7.6, 10 mM MgCl₂, 5 mM dithiothreitol (DTT), 10 mM beta-mercaptoethanol, 500 micrograms per milliliter (μ g/ml) bovine serum albumin (BSA). The solution was maintained at 37 degrees Centigrade (37°C) for 30 minutes and the reaction stopped by maintaining the solution at 65°C for 10 minutes. The two end polynucleotides, 20 nanograms (ng) of polynucleotides N1 and polynucleotides N12, were added to the above kinasing reaction solution together with 1/10 volume of a solution containing 20 mM Tris-HCl at pH 7.4, 2.0 mM MgCl₂ and 50 mM NaCl. This solution was heated to 70°C for 5 minutes and

allowed to cool to room temperature, approximately 25°C, over 1.5 hours in a 500 ml beaker of water. During this time period all 10 polynucleotides annealed to form the double stranded synthetic DNA insert shown in Figure 1.

5 The individual polynucleotides were covalently linked to each other to stabilize the synthetic DNA insert by adding 40 μ l of the above reaction to a solution containing 50 mM Tris-HCl at pH 7.5, 7 mM $MgCl_2$, 1 mM DTT, 1 mM adenosine triphosphate (ATP) and 10 units of T4 DNA ligase. This

10 solution was maintained at 37°C for 30 minutes and then the T4 DNA ligase was inactivated by maintaining the solution at 65°C for 10 minutes. The end polynucleotides were kinased by mixing 52 μ l of the above reaction, 4 μ l of a solution containing 10 mM ATP and 5 units of T4

15 polynucleotide kinase. This solution was maintained at 37°C for 30 minutes and then the T4 polynucleotide kinase was inactivated by maintaining the solution at 65°C for 10 minutes.

20

Table 1

SEQ			
<u>ID NO</u>			
25	(15)	N1)	5' GGCCGCAAATTCTATTTCAAGGAGACAGTCAT 3'
	(16)	N2)	5' AATGAAATACCTATTGCCTACGGCAGCCGCTGGATT 3'
	(17)	N3)	5' GTTATTACTCGCTGCCCCAACCAGCCATGGCCC 3'
	(18)	N6)	5' CAGTTTCACCTGGGCCATGGCTGGTTGGG 3'
	(19)	N7)	5' CAGCGAGTAATAACAATCCAGCGGCTGCCGTAGGCAATAG 3'
30	(20)	N8)	5' GTATTTTCATTATGACTGTCTCCTTGAAATAGAATTGTC 3'
	(21)	N9-4)	5' AGGTGAAACTGCTCGAGATTTCTAGACTAGTTACCCGTAC 3'
	(22)	N10-5)	5' CGGAACGTCGTACGGGTAAGTCTAGAAATCTCGAG 3'
	(23)	N11)	5' GACGTTCCGGACTACGGTTCTTAATAGAATTTCG 3'
	(24)	N12)	5' TCGACGAATTCTATTAAGAACCGTAGTC 3'

35

5

The completed synthetic DNA insert was ligated directly into the Lambda ZapTM II vector described in Example 1a1) that had been previously digested with the restriction enzymes, Not I and Xho I. The ligation mixture was packaged according to the manufacture's instructions using Gigapack II Gold packing extract available from Stratagene, La

Jolla, California. The packaged ligation mixture was plated on XL1-Blue cells (Stratagene). Individual lambda plaques were cored and the inserts excised according to the in vivo excision protocol for Lambda Zap™ II provided by the manufacturer (Stratagene). This in vivo excision protocol moved the cloned insert from the Lambda Hc2 vector into a phagemid vector to allow easy for manipulation and sequencing. The accuracy of the above cloning steps was confirmed by sequencing the insert using the Sanger dideoxy method described in by Sanger et al., Proc. Natl. Acad. Sci., USA, 74:5463-5467 (1977) and using the manufacture's instructions in the AMV Reverse Transcriptase ³⁵S-ATP sequencing kit (Stratagene). The sequence of the resulting double-stranded synthetic DNA insert in the V_H expression vector (Lambda Hc2) is shown in Figure 1. The sequence of each strand (top and bottom) of Lambda Hc2 is listed in the Sequence Listing as SEQ ID NO 1 and SEQ ID NO 2, respectively. The resultant Lambda Hc2 expression vector is shown in Figure 2.

3) Preparation of Lambda Lc2

To express a plurality of V_L-coding DNA homologs in an E. coli host cell, a vector designated Lambda Lc2 was constructed having the capacity to place the V_L-coding DNA homologs in the proper reading frame, provided a ribosome binding site as described by Shine et al., Nature, 254:34 (1975), provided the pelB gene leader sequence secretion signal that has been previously used to successfully secrete Fab fragments in E. coli by Lei et al., J. Bac., 169:4379 (1987) and Better et al., Science, 240:1041 (1988), and also provided a polynucleotide containing a restriction endonuclease site for cloning. Lambda Lc2 has been previously described by Huse et al., Science,

246:1275-1281 (1989).

A synthetic DNA sequence containing all of the above features was constructed by designing single stranded polynucleotide segments of 20-60 bases that would hybridize to each other and form the double stranded synthetic DNA sequence shown in Figure 3. The sequence of each individual single-stranded polynucleotide segment (01-08) within the double stranded synthetic DNA sequence is shown in Table 2.

Polynucleotides 02, 03, 04, 05, 06 and 07 (Table 2) were kinased by adding 1 μ l (0.1 μ g/ μ l) of each polynucleotide and 20 units of T_4 polynucleotide kinase to a solution containing 70 mM Tris-HCl at pH 7.6, 10 mM $MgCl_2$, 5 mM DTT, 10 mM beta-mercaptoethanol, 500 μ g/ml of BSA. The solution was maintained at 37°C for 30 minutes and the reaction stopped by maintaining the solution at 65°C for 10 minutes. The 20 ng each of the two end polynucleotides, 01 and 08, were added to the above kinasing reaction solution together with 1/10 volume of a solution containing 20.0 mM Tris-HCl at pH 7.4, 2.0 mM $MgCl_2$ and 15.0 mM sodium chloride (NaCl). This solution was heated to 70°C for 5 minutes and allowed to cool to room temperature, approximately 25°C, over 1.5 hours in a 500 ml beaker of water. During this time period all 8 polynucleotides annealed to form the double stranded synthetic DNA insert shown in Figure 3. The individual polynucleotides were covalently linked to each other to stabilize the synthetic DNA insert by adding 40 μ l of the above reaction to a solution containing 50 mM Tris-HCl at pH 7.5, 7 mM $MgCl_2$, 1 mM DTT, 1 mM ATP and 10 units of T_4 DNA ligase. This solution was maintained at 37°C for 30 minutes and then the T_4 DNA ligase was inactivated by maintaining the solution at 65°C for 10 minutes. The end polynucleotides were kinased

by mixing 52 μ l of the above reaction, 4 μ l of a solution containing 10 mM ATP and 5 units of T4 polynucleotide kinase. This solution was maintained at 37°C for 30 minutes and then the T4 polynucleotide kinase was inactivated by maintaining the solution at 65°C for 10 minutes.

Table 2

SEQ	ID NO	
(25)	01)	5' TGAATTCTAAACTAGTCGCCAAGGAGACAGTCAT 3'
(26)	02)	5' AATGAAATACCTATTGCCTACGGCAGCCGCTGGATT 3'
(27)	03)	5' GTTATTACTCGCTGCCCAACCAGCCATGGCC 3'
(28)	04)	5' GAGCTCGTCAGTTCTAGAGTTAAGCGGCCG 3'
(29)	05)	5' GTATTTCAATTATGACTGTCTCCTTGGCGACTAGTTTAGAA- TTCAAGCT 3'
(30)	06)	5' CAGCGAGTAATAACAATCCAGCGGCTGCCGTAGGCAATAG 3'
(31)	07)	5' TGACGAGCTCGGCCATGGCTGGTTGGG 3'
(32)	08)	5' TCGACGGCCGCTTAACTCTAGAAC 3'

The completed synthetic DNA insert was ligated directly into the Lambda Zap™ II vector described in Example 1a) that had been previously digested with the restriction enzymes Sac I and Xho I. The ligation mixture was packaged according to the manufacture's instructions using Gigapack II Gold packing extract (Stratagene). The packaged ligation mixture was plated on XL1-Blue cells (Stratagene). Individual lambda plaques were cored and the inserts excised according to the in vivo excision protocol for Lambda Zap™ II provided by the manufacturer (Stratagene). This in vivo excision protocol moved the cloned insert from the Lambda Lc2 vector into a plasmid phagemid vector allow for easy manipulation and sequencing. The accuracy of the above cloning steps was confirmed

by sequencing the insert using the manufacture's instructions in the AMV Reverse Transcriptase ³⁵S-dATP sequencing kit (Stratagene). The sequence of the resulting Lc2 expression vector (Lambda Lc2) is shown in Figure 3. Each strand is separately listed in the Sequence Listing as SEQ ID NO 3 and SEQ ID NO 4. The resultant Lc2 vector is schematically diagrammed in Figure 4.

A preferred vector for use in this invention, designated Lambda Lc3, is a derivative of Lambda Lc2 prepared above. Lambda Lc2 contains a Spe I restriction site located 3' to the EcoR I restriction site and 5' to the Shine-Dalgarno ribosome binding site as shown in the sequence in Figure 3 and in SEQ ID NO 3. A Spe I restriction site is also present in Lambda Hc2 as shown in Figures 1 and 2 and in SEQ ID NO 1. A combinatorial vector, designated pComb, was constructed by combining portions of Lambda Hc2 and Lc2 together as described in Example 1a4) below. The resultant combinatorial pComb vector contained two Spe I restriction sites, one provided by Lambda Hc2 and one provided by Lambda Lc2, with an EcoR I site in between. Despite the presence of two Spe I restriction sites, DNA homologs having Spe I and EcoR I cohesive termini were successfully directionally ligated into a pComb expression vector previously digested with Spe I and EcoR I as described in Example 1b below. The proximity of the EcoR I restriction site to the 3' Spe I site, provided by the Lc2 vector, inhibited the complete digestion of the 3' Spe I site. Thus, digesting pComb with Spe I and EcoR I did not result in removal of the EcoR I site between the two Spe I sites.

The presence of a second Spe I restriction site may be undesirable for ligations into a pComb vector digested only with Spe I as the region

between the two sites would be eliminated.

Therefore, a derivative of Lambda Lc2 lacking the second or 3' Spe I site, designated Lambda Lc3, was produced by first digesting Lambda Lc2 with Spe I to form a linearized vector. The ends were filled in to form blunt ends which are ligated together to result in Lambda Lc3 lacking a Spe I site. Lambda Lc3 is a preferred vector for use in constructing a combinatorial vector as described below.

4) Preparation of pComb

Phagemids were excised from the expression vectors Lambda Hc2 or Lambda Lc2 using an in vivo excision protocol described above. Double stranded DNA was prepared from the phagemid-containing cells according to the methods described by Holmes et al., Anal. Biochem., 114:193 (1981). The phagemids resulting from in vivo excision contained the same nucleotide sequences for antibody fragment cloning and expression as did the parent vectors, and are designated phagemid Hc2 and Lc2, corresponding to Lambda Hc2 and Lc2, respectively.

For the construction of combinatorial phagemid vector pComb, produced by combining portions of phagemid Hc2 and phagemid Lc2, phagemid Hc2 was first digested with Sac I to remove the restriction site located 5' to the LacZ promoter. The linearized phagemid was then blunt ended with T4 polymerase and ligated to result in a Hc2 phagemid lacking a Sac I site. The modified Hc2 phagemid and the Lc2 phagemid were then separately restriction digested with Sca I and EcoR I to result in a Hc2 fragment having from 5' to 3' Sca I, Not I, Xho I, Spe I and EcoR I restriction sites and a Lc2 fragment having from 5' to 3' EcoR I, Sac I, Xba I and Sac I restriction sites. The linearized phagemids were then ligated together at

their respective cohesive ends to form pComb, a circularized phagemid having a linear arrangement of restriction sites of Not I, Xho I, Spe I, EcoR I, Sac I, Xba I, Not I, Apa I and Sca I. The ligated phagemid vector was then inserted into an appropriate bacterial host and transformants were selected on the antibiotic ampicillin.

Selected ampicillin resistant transformants were screened for the presence of two Not I sites. The resulting ampicillin resistant combinatorial phagemid vector was designated pComb, the schematic organization of which is shown in Figure 5. The resultant combinatorial vector, pComb, consisted of a DNA molecule having two cassettes to express two fusion proteins and having nucleotide residue sequences for the following operatively linked elements listed in a 5' to 3' direction: a first cassette consisting of an inducible LacZ promoter upstream from the LacZ gene; a Not I restriction site; a ribosome binding site; a pelB leader; a spacer; a cloning region bordered by a 5' Xho and 3' Spe I restriction site; a decapeptide tag followed by expression control stop sequences; an EcoR I restriction site located 5' to a second cassette consisting of an expression control ribosome binding site; a pelB leader; a spacer region; a cloning region bordered by a 5' Sac I and a 3' Xba I restriction site followed by expression control stop sequences and a second Not I restriction site.

A preferred combinatorial vector for use in this invention, designated pComb2, is constructed by combining portions of phagemid Hc2 and phagemid Lc3 as described above for preparing pComb. The resultant combinatorial vector, pComb2, consists of a DNA molecule having two cassettes identical to pComb to express two fusion proteins identically to pComb except that a second Spe I restriction site

in the second cassette is eliminated.

b. Construction of the pCombIII Vector for
Expressing Fusion Proteins Having a
Bacteriophage Coat Protein Membrane
Anchor

Because of the multiple endonuclease restriction cloning sites, the pComb phagemid expression vector prepared above is a useful cloning vehicle for modification for the preparation of an expression vector for use in this invention. To that end, pComb was digested with EcoR I and Spe I followed by phosphatase treatment to produce linearized pComb.

1) Preparation of pCombIII

A separate phagemid expression vector was constructed using sequences encoding bacteriophage cpIII membrane anchor domain. A PCR product defining the DNA sequence encoding the filamentous phage coat protein, cpIII, membrane anchor containing a LacZ promotor region sequence 3' to the membrane anchor for expression of the light chain and Spe I and EcoR I cohesive termini was prepared from M13mp18, a commercially available bacteriophage vector (Pharmacia, Piscataway, New Jersey).

To prepare a modified cpIII, replicative form DNA from M13mp18 was first isolated. Briefly, into 2 ml of LB (Luria-Bertani medium), 50 μ l of a culture of a bacterial strain carrying an F' episome (JM107, JM109 or TG1) was admixed with a one tenth suspension of bacteriophage particles derived from a single plaque. The admixture was incubated for 4 to 5 hours at 37°C with constant agitation. The admixture was then centrifuged at 12,000 x g for 5 minutes to pellet the infected bacteria. After the supernatant was removed, the

pellet was resuspended by vigorous vortexing in 100 μ l of ice-cold solution I. Solution I was prepared by admixing 50 mM glucose, 10 mM EDTA (disodium ethylenediaminetetraacetic acid) and 25 mM Tris-HCl at pH 8.0, and autoclaving for 15 minutes.

To the bacterial suspension, 200 μ l of freshly prepared Solution II was admixed and the tube was rapidly inverted five times. Solution II was prepared by admixing 0.2 N NaOH and 1% SDS. To the bacterial suspension, 150 μ l of ice-cold Solution III was admixed and the tube was vortexed gently in an inverted position for 10 seconds to disperse Solution III through the viscous bacterial lysate. Solution III was prepared by admixing 60 ml of 5 M potassium acetate, 11.5 ml of glacial acetic acid and 28.5 ml of water. The resultant bacterial lysate was then stored on ice for 5 minutes followed by centrifugation at 12,000 x g for 5 minutes at 4°C in a microfuge. The resultant supernatant was recovered and transferred to a new tube. To the supernatant was added an equal volume of phenol/chloroform and the admixture was vortexed. The admixture was then centrifuged at 12,000 x g for 2 minutes in a microfuge. The resultant supernatant was transferred to a new tube and the double-stranded bacteriophage DNA was precipitated with 2 volumes of ethanol at room temperature. After allowing the admixture to stand at room temperature for 2 minutes, the admixture was centrifuged to pellet the DNA. The supernatant was removed and the pelleted replicative form DNA was resuspended in 25 μ l of Tris-HCl at pH 7.6, and 10 mM EDTA (TE).

An alternative Lac-B primer for use in constructing the cpIII membrane anchor and LacZ promotor region was Lac-B' as shown in Table 3. The amplification reactions were performed as described above with the exception that in the

second PCR amplification, Lac-B' was used with Lac-F instead of Lac-B. The product from the amplification reaction is listed in the sequence listing as SEQ ID NO 41 from nucleotide position 1 to nucleotide position 172. The use of Lac-B' resulted in a LacZ region lacking 29 nucleotides on the 3' end but was functionally equivalent to the longer fragment produced with the Lac-F and Lac-B primers.

The products of the first and second PCR amplifications using the primer pairs G-3(F) and G-3(B) and Lac-F and Lac-B were then recombined at the nucleotides corresponding to cpIII membrane anchor overlap and Nhe I restriction site and subjected to a second round of PCR using the G-3(F) (SEQ ID NO 35) and Lac-B (SEQ ID NO 38) primer pair to form a recombined PCR DNA fragment product consisting of the following: a 5' Spe I restriction site; a cpIII DNA membrane anchor domain beginning at the nucleotide residue sequence which corresponds to the amino acid residue 198 of the entire mature cpIII protein; an endogenous stop site provided by the membrane anchor at amino acid residue number 112; a Nhe I restriction site, a LacZ promoter, operator and Cap-binding site sequence; and a 3' EcoR I restriction site.

To construct a phagemid vector for the coordinate expression of a heavy chain-cpIII fusion protein as prepared in Example 2 with kappa light chain, the recombined PCR modified cpIII membrane anchor domain DNA fragment was then restriction digested with Spe I and EcoR I to produce a DNA fragment for directional ligation into a similarly digested pComb2 phagemid expression vector having only one Spe I site prepared in Example 1a4) to form a pComb2-III (also referred to as pComb2-III) phagemid expression vector. Thus, the resultant ampicillin resistance conferring pComb2-3 vector,

having only one Spe I restriction site, contained separate LacZ promoter/operator sequences for directing the separate expression of the heavy chain (Fd)-cpIII fusion product and the light chain protein. The expressed proteins were directed to the periplasmic space by pelB leader sequences for functional assembly on the membrane. Inclusion of the phage F1 intergenic region in the vector allowed for packaging of single stranded phagemid with the aid of helper phage. The use of helper phage superinfection lead to expression of two forms of cpIII. Thus, normal phage morphogenesis was perturbed by competition between the Fab-cpIII fusion and the native cpIII of the helper phage for incorporation into the virion for Fab-cpVIII fusions. In addition, also contemplated for use in this invention are vectors conferring chloramphenicol resistance and the like.

A more preferred phagemid expression vector for use in this invention having additional restriction enzyme cloning sites, designated pComb-III' or pComb2-3', was prepared as described above for pComb2-3 with the addition of a 51 base pair fragment from pBluescript as described by Short et al., Nuc. Acids Res., 16:7583-7600 (1988) and commercially available from Stratagene. To prepare pComb2-3', pComb2-3 was first digested with Xho I and Spe I restriction enzymes to form a linearized pComb2-3. The vector pBluescript was digested with the same enzymes releasing a 51 base pair fragment containing the restriction enzyme sites Sal I, Acc I, Hinc II, Cla I, Hind III, EcoR V, Pst I, Sma I and BamH I. The 51 base pair fragment was ligated into the linearized pComb2-3 vector via the cohesive Xho I and Spe I termini to form pComb2-3'.

Table 3

SEQ					
ID NO	Primer				
5	(35) ¹	G-3 (F)	5'	<u>GAGACGACTAGTGGTGGCGGTGGCTCTCCATTC</u> <u>GTTTGTGAATATCAA</u> 3'	
	(36) ²	G-3 (B)	5'	<u>TTACTAGCTAGCATAATAACGGAATACCCAAAA</u> <u>GAACTGG</u> 3'	
	(37) ³	LAC-F	5'	<u>TATGCTAGCTAGTAA</u> CACGACAGGTTTCCCGAC TGG 3'	
10	(38) ⁴	LAC-B	5'	ACCGAGCTCGAATTCGTAATCATGGTC 3'	
	(39) ⁵	LAC-B'	5'	AGCTGTTGAATTCGTGAAATTGTTATCCGCT 3'	

F Forward Primer

15 B Backward Primer

1 From 5' to 3': Spe I restriction site sequence is single underlined; the overlapping sequence with the 5' end of cpIII is double underlined

20 2 From 5' to 3': Nhe I restriction site sequence is single underlined; the overlapping sequence with 3' end of cpIII is double underlined.

25 3 From 5' to 3': overlapping sequence with the 3' end of cpIII is double underlined; Nhe I restriction sequence begins with the nucleotide residue "G" at position 4 and extends 5 more residues = GCTAGC.

4 EcoR I restriction site sequence is single underlined.

30 5 Alternative backwards primer for amplifying LacZ; EcoR I restriction site sequence is single underlined.

35 2. Isolation of HIV-1-Specific Monoclonal Antibodies Produced from the Dicistronic Expression Vector, pComb2-3

In practicing this invention, the heavy (Fd

consisting of V_H and C_H1) and light (κ) chains (V_L , C_L) of antibodies are first targeted to the periplasm of E. coli for the assembly of heterodimeric Fab molecules. In order to obtain expression of antibody Fab libraries on a phage surface, the nucleotide residue sequences encoding either the Fd or light chains must be operatively linked to the nucleotide residue sequence encoding a filamentous bacteriophage coat protein membrane anchor. A coat protein for use in this invention in providing a membrane anchor is III (cpIII or cp3). In the Examples described herein, methods for operatively linking a nucleotide residue sequence encoding a Fd chain to a cpIII membrane anchor in a fusion protein of this invention are described.

In a phagemid vector, a first and second cistron consisting of translatable DNA sequences are operatively linked to form a dicistronic DNA molecule. Each cistron in the dicistronic DNA molecule is linked to DNA expression control sequences for the coordinate expression of a fusion protein, Fd-cpIII, and a kappa light chain.

The first cistron encodes a periplasmic secretion signal (pelB leader) operatively linked to the fusion protein, Fd-cpIII. The second cistron encodes a second pelB leader operatively linked to a kappa light chain. The presence of the pelB leader facilitates the coordinated but separate secretion of both the fusion protein and light chain from the bacterial cytoplasm into the periplasmic space.

In this process, the phagemid expression vector carries an ampicillin selectable resistance marker gene (beta lactamase or bla) in addition to the Fd-cpIII fusion and the kappa chain. The f1 phage origin of replication facilitates the generation of single stranded phagemid. The

isopropyl thiogalactopyranoside (IPTG) induced expression of a dicistronic message encoding the Fd-cpIII fusion (V_H , C_H , cpIII) and the light chain (V_L , C_L) leads to the formation of heavy and light chains. Each chain is delivered to the periplasmic space by the pelB leader sequence, which is subsequently cleaved. The heavy chain is anchored in the membrane by the cpIII membrane anchor domain while the light chain is secreted into the periplasm. The heavy chain in the presence of light chain assembles to form Fab molecules. This same result can be achieved if, in the alternative, the light chain is anchored in the membrane via a light chain fusion protein having a membrane anchor and heavy chain is secreted via a pelB leader into the periplasm.

With subsequent infection of E. coli with a helper phage, as the assembly of the filamentous bacteriophage progresses, the coat protein III is incorporated on the tail of the bacteriophage.

a. Preparation of Lymphocyte RNA

Five milliliters of bone marrow was removed by aspiration from HIV-1 asymptomatic seropositive individuals. Total cellular RNA was prepared from the bone marrow lymphocytes as described above using the RNA preparation methods described by Chomczynski et al., Anal Biochem., 162:156-159 (1987) and using the RNA isolation kit (Stratagene) according to the manufacturer's instructions. Briefly, for immediate homogenization of the cells in the isolated bone marrow, 10 ml of a denaturing solution containing 3.0 M guanidinium isothiocyanate containing 71 μ l of beta-mercaptoethanol was admixed to the isolated bone marrow. One ml of sodium acetate at a concentration of 2 M at pH 4.0 was then admixed with the homogenized cells. One ml of phenol that

had been previously saturated with H_2O was also admixed to the denaturing solution containing the homogenized spleen. Two ml of a chloroform:isoamyl alcohol (24:1 v/v) mixture was added to this homogenate. The homogenate was mixed vigorously for ten seconds and maintained on ice for 15 minutes. The homogenate was then transferred to a thick-walled 50 ml polypropylene centrifuged tube (Fisher Scientific Company, Pittsburgh, PA). The solution was centrifuged at $10,000 \times g$ for 20 minutes at $4^\circ C$. The upper RNA-containing aqueous layer was transferred to a fresh 50 ml polypropylene centrifuge tube and mixed with an equal volume of isopropyl alcohol. This solution was maintained at $-20^\circ C$ for at least one hour to precipitate the RNA. The solution containing the precipitated RNA was centrifuged at $10,000 \times g$ for twenty minutes at $4^\circ C$. The pelleted total cellular RNA was collected and dissolved in 3 ml of the denaturing solution described above. Three ml of isopropyl alcohol was added to the re-suspended total cellular RNA and vigorously mixed. This solution was maintained at $-20^\circ C$ for at least 1 hour to precipitate the RNA. The solution containing the precipitated RNA was centrifuged at $10,000 \times g$ for ten minutes at $4^\circ C$. The pelleted RNA was washed once with a solution containing 75% ethanol. The pelleted RNA was dried under vacuum for 15 minutes and then re-suspended in dimethyl pyrocarbonate-treated (DEPC- H_2O) H_2O .

Messenger RNA (mRNA) enriched for sequences containing long poly A tracts was prepared from the total cellular RNA using methods described in Molecular Cloning: A Laboratory Manual, Maniatis et al., eds., Cold Spring Harbor, NY, (1982). Briefly, one half of the total RNA isolated from a single donor prepared as described above was resuspended in one ml of DEPC- H_2O and maintained at

65°C for five minutes. One ml of 2X high salt loading buffer consisting of 100 mM Tris-HCl, 1 M NaCl, 2.0 mM EDTA at pH 7.5, and 0.2% SDS was admixed to the resuspended RNA and the mixture allowed to cool to room temperature.

The total purified mRNA was then used in PCR amplification reactions as described in Example 2c. Alternatively, the mRNA was further purified to poly A+ RNA by the following procedure. The total mRNA was applied to an oligo-dT (Collaborative Research Type 2 or Type 3) column that was previously prepared by washing the oligo-dT with a solution containing 0.1 M sodium hydroxide and 5 mM EDTA and then equilibrating the column with DEPC-H₂O. The eluate was collected in a sterile polypropylene tube and reapplied to the same column after heating the eluate for 5 minutes at 65°C. The oligo-dT column was then washed with 2 ml of high salt loading buffer consisting of 50 mM Tris-HCl at pH 7.5, 500 mM sodium chloride, 1 mM EDTA at pH 7.5 and 0.1% SDS. The oligo dT column was then washed with 2 ml of 1X medium salt buffer consisting of 50 mM Tris-HCl, pH 7.5, 100 mM, 1 mM EDTA and 0.1% SDS. The messenger RNA was eluted from the oligo-dT column with 1 ml of buffer consisting of 10 mM Tris-HCl at pH 7.5, 1 mM EDTA at pH 7.5, and 0.05% SDS. The messenger RNA was purified by extracting this solution with phenol/chloroform followed by a single extraction with 100% chloroform. The messenger RNA was concentrated by ethanol precipitation and resuspended in DEPC H₂O.

The resultant purified mRNA contained a plurality of anti-HIV encoding V_H and V_L sequences for preparation of an anti-HIV-1 Fab DNA library.

b. Construction of a Combinatorial HIV-1
Antibody Library

1) Selection of Oligonucleotide Primers

The nucleotide sequences encoding
5 the immunoglobulin protein CDR's are highly
variable. However, there are several regions of
conserved sequences that flank the V region domains
of either the light or heavy chain, for instance,
and that contain substantially conserved nucleotide
10 sequences, i.e., sequences that will hybridize to
the same primer sequence. Therefore,
polynucleotide synthesis (amplification) primers
that hybridize to the conserved sequences and
incorporate restriction sites into the DNA homolog
15 produced that are suitable for operatively linking
the synthesized DNA fragments to a vector were
constructed. More specifically, the primers were
designed so that the resulting DNA homologs
produced can be inserted into an expression vector
20 of this invention in reading frame with the
upstream translatable DNA sequence at the region of
the vector containing the directional ligation
means.

For amplification of the V_H domains, primers
25 were designed to introduce cohesive termini
compatible with directional ligation into the
unique Xho I and Spe I sites of the pComb2-3
expression vector. In all cases, the 5' primers
VH1a (5' CAGGTGCAGCTCGAGCAGTCTGGG 3' SEQ ID NO 42)
30 and VH3a (5' GAGGTGCAGCTCGAGGAGTCTGGG 3' SEQ ID NO
43) were designed to maximize homology with the V_H1
and V_H3 subgroup families, respectively, although
considerable cross-priming of other subgroups was
expected. The Xho I restriction site for cloning
35 into the pComb2-3 vector is underlined. The 3'
primer CG1z having the nucleotide sequence 5'
GCATGTACTAGTTTTGTCACAAGATTGGG 3' (SEQ ID NO 44)
used in conjunction with the 5' primers is the

primer for the heavy chain corresponding to part of the hinge region. The Spe I site for cloning into the pComb2-3 vector is underlined.

5 The nucleotide sequences encoding the V_L domain are highly variable. However, there are several regions of conserved sequences that flank the V_L domains including the J_L , V_L framework regions and V_L leader/promotor. Therefore, amplification primers were constructed that hybridized to the
10 conserved sequences and incorporate restriction sites that allow cloning the amplified fragments into the pComb2-3 expression vector cut with Sac I and Xba I.

15 For amplification of the kappa V_L domains analogous to the heavy chain primers listed above, the 5' primers, VK1a (5' GACATCGAGCTCACCAGTCTCCA 3' SEQ ID NO 45) and VK3a (5' GAAATTGAGCTCACGCAGTCTCCA 3' SEQ ID NO 46), were
20 used. These primers also introduced a Sac I restriction endonuclease site indicated by the underlined nucleotides to allow the V_L DNA homolog to be cloned into the pComb2-3 expression vector. The 3' V_L amplification primer, CK1a having a
25 nucleotide sequence 5' GCGCCGTCCTAGAACTAACACTCTCCCCTGTTGAAGCTCTTTGTGACGGGCA AG 3' (SEQ ID NO 47) corresponding to the 3' end of the light chain was used to amplify the light chain while incorporating the underlined Xba I
30 restriction endonuclease site required to insert the V_L DNA homolog into the pComb2-3 expression vector.

35 All primers and synthetic polynucleotides described herein, were either purchased from Research Genetics in Huntsville, Alabama or synthesized on an Applied Biosystems DNA synthesizer, model 381A, using the manufacturer's instruction.

2) PCR Amplification of V_H and V_L DNA
Homologs

In preparation for PCR

amplification, mRNA prepared above was used as a
template for cDNA synthesis by a primer extension
reaction. First, 20-50 µg of total mRNA in water
was first hybridized (annealed) at 70°C for 10
minutes with 600 ng (60.0 pmol) of either the heavy
or light chain 3' primers listed above.

Subsequently, the hybridized admixture was used in
a typical 50 µl reverse transcription reaction
containing 200 µM each of dATP, dCTP, dGTP and
dTTP, 40 mM Tris-HCl at pH 8.0, 8 mM MgCl₂, 50 mM
NaCl, 2 mM spermidine and 600 units of reverse
transcriptase (SuperScript, BRL). The reaction
admixture was then maintained for one hour at 37°C
to form an RNA-cDNA admixture.

Three µl of the resultant RNA-cDNA admixture
was then used in PCR amplification in a reaction
volume of 100 µl containing a mixture of all four
dNTPs at a concentration of 60 µM, 50 mM KCl, 10 mM
Tris-HCl at pH 8.3, 15 mM MgCl₂, 0.1% gelatin and 5
units of Thermus aquaticus (Taq) DNA polymerase
(Perkin-Elmer-Cetus, Emeryville, California), and
60 pmol of the appropriate 5' and 3' primers listed
above. The separate reaction admixtures were
overlaid with mineral oil and subjected to 35
cycles of amplification. Each amplification cycle
included denaturation at 91°C for 1 minute,
annealing at 52°C for 2 minutes and polynucleotide
synthesis by primer extension (elongation) at 72°C
for 1.5 minutes, followed by a final maintenance
period of 10 minutes at 72°C. An aliquot of the
reaction admixtures were then separately
electrophoresed on a 2% agarose gel. After
successful amplification as determined by gel
electrophoretic migration, the remainder of the
RNA-cDNA was amplified after which the PCR products

of a common 3' primer were pooled into separate V_H -and V_L -coding DNA homolog-containing samples and were then extracted twice with phenol/chloroform, once with chloroform, ethanol precipitated and were stored at -70°C in 10 mM Tris-HCl at pH 7.5, and 1 mM EDTA.

3) Insertion of V_H and V_L -Coding DNA Homologs into pComb2-3 Expression Vector

The V_H -coding DNA homologs (heavy chain) prepared above were then digested with an excess of Xho I and Spe I for subsequent ligation into a similarly digested and linearized pComb2-3 in a total volume of 150 μl with 10 units of ligase at 16°C overnight. The construction of the library was performed as described by Burton et al., Proc. Natl. Acad. Sci., USA, 88:10134-10137 (1991). Briefly, following ligation, the pComb2-3 vector containing heavy chain DNA was then transformed by electroporation into 300 μl of XL1-Blue cells. After transformation and culturing, library size was determined by plating aliquots of the culture. Typically the library had about 10^7 members. An overnight culture was then prepared from which phagemid DNA containing the heavy chain library was prepared.

For the cloning of the V_L -coding DNA homologs (light chain), 10 μg of phagemid DNA containing the heavy chain library was then digested with Sac I and SbaI. The resulting linearized vector was treated with phosphatase and purified by agarose gel electrophoresis. The desired fragment, 4.7 kb in length, was excised from the gel. Ligation of this vector with prepared light chain PCR DNA proceeded as described above for heavy chain. A library of approximately 10^7 members having heavy chain fragments operatively linked to the cpIII

anchor sequence (Fd-cpIII) and light chain fragments was thus produced.

4) Preparation of Phage Expressing Fab Heterodimers

Following transformation of the resultant library produced above into XL1-Blue cells, phage were prepared to allow for isolation of HIV-1 specific Fabs by panning on target antigens. To isolate phage on which heterodimer expression has been induced, 3 ml of SOC medium (SOC was prepared by admixture of 20 g bacto-tryptone, 5 g yeast extract and 0.5 g NaCl in one liter of water, adjusting the pH to 7.5 and admixing 20 ml of glucose just before use to induce the expression of the Fd-cpIII and light chain heterodimer) was admixed and the culture was shaken at 220 rpm for one hour at 37°C, after which time 10 ml of SB (SB was prepared by admixing 30 g tryptone, 20 g yeast extract, and 10 g Mops buffer per liter with pH adjusted to 7) containing 20 µg/ml carbenicillin and 10 µg/ml tetracycline and the admixture was shaken at 300 rpm for an additional hour. This resultant admixture was admixed to 100 ml SB containing 50 µg/ml carbenicillin and 10 µg/ml tetracycline and shaken for one hour, after which time helper phage VCSM13 (10^{12} pfu) were admixed and the admixture was shaken for an additional two hours. After this time, 70 µg/ml kanamycin was admixed and maintained at 30°C overnight. The lower temperature resulted in better heterodimer incorporation on the surface of the phage. The supernatant was cleared by centrifugation (4000 rpm for 15 minutes in a JA10 rotor at 4°C). Phage were precipitated by admixture of 4% (w/v) polyethylene glycol 8000 and 3% (w/v) NaCl and maintained on ice for 30 minutes, followed by centrifugation (9000 rpm for 20 minutes

5 in a JA10 rotor at 4°C). Phage pellets were resuspended in 2 ml of PBS and microcentrifuged for three minutes to pellet debris, transferred to fresh tubes and stored at -20°C for subsequent screening as described below.

For determining the titering colony forming units (cfu), phage (packaged phagemid) were diluted in SB and 1 µl was used to infect 50 µl of fresh (OD600 = 1) XL1-Blue cells grown in SB containing 10 µg/ml tetracycline. Phage and cells were maintained at room temperature for 15 minutes and then directly plated on LB/carbenicillin plates.

15 5) Selection of Anti-HIV-1 Heterodimers on Phage Surfaces

(a) Multiple Pannings of the Phage Library

The phage library produced in Example 2b4) was panned against recombinant gp120 of HIV-1 strain IIIb as described herein on coated microtiter plate to select for anti-gp120 heterodimers. A second phage library was panned against recombinant gp41 (American Biotechnologies, Boston, MA) as described below to select for anti-gp41 heterodimers.

The panning procedure used was a modification of that originally described by Parmley and Smith (Parmley et al., Gene, 73:305-318 (1988)). Four rounds of panning were performed to enrich for specific antigen-binding clones. For this procedure, four wells of a microtiter plate (Costar 3690) were coated overnight at 4°C with 25 µl of 40 µg/ml gp120 or gp41 (American Biotechnologies) prepared above in 0.1 M bicarbonate, pH 8.6. The wells were washed twice with water and blocked by completely filling the well with 3% (w/v) BSA in PBS and maintaining the plate at 37°C for one hour. After the blocking solution was shaken out, 50 µl

of the phage library prepared above (typically 10^{11} cfu) were admixed to each well, and the plate was maintained for two hours at 37°C .

5 Phage were removed and the plate was washed once with water. Each well was then washed ten times with TBS/Tween (50 mM Tris-HCl at pH 7.5, 150 mM NaCl, 0.5% Tween 20) over a period of one hour at room temperature where the washing consisted of
10 pipetting up and down to wash the well, each time allowing the well to remain completely filled with TBS/Tween between washings. The plate was washed once more with distilled water and adherent phage were eluted by the addition of 50 μl of elution
15 buffer (0.1 M HCl, adjusted to pH 2.2 with solid glycine, containing 1 mg/ml BSA) to each well followed by maintenance at room temperature for 10 minutes. The elution buffer was pipetted up and down several times, removed, and neutralized with 3
20 μl of 2 M Tris base per 50 μl of elution buffer used.

Eluted phage were used to infect 2 ml of fresh ($\text{OD}_{600} = 1$) E. coli XL1-Blue cells for 15 minutes at room temperature, after which time 10 ml of SB containing 20 $\mu\text{g/ml}$ carbenicillin and 10 $\mu\text{g/ml}$
25 tetracycline was admixed. Aliquots of 20, 10, and 1/10 μl were removed from the culture for plating to determine the number of phage (packaged phagemids) that were eluted from the plate. The culture was shaken for one hour at 37°C , after
30 which it was added to 100 ml of SB containing 50 $\mu\text{g/ml}$ carbenicillin and 10 $\mu\text{g/ml}$ tetracycline and shaken for one hour. Helper phage VCSM13 (10^{12} pfu) were then added and the culture was shaken for an additional two hours. After this time, 70 $\mu\text{g/ml}$
35 kanamycin was added and the culture was incubated at 37°C overnight. Phage preparation and further panning were repeated as described above.

Following each round of panning, the percentage yield of phage were determined, where % yield - (number of phage eluted/number of phage applied) X 100. The initial phage input ratio was determined by titring on selective plates to be approximately 10^{11} cfu for each round of panning. The final phage output ratio was determined by infecting two ml of logarithmic phase XL1-Blue cells as described above and plating aliquots on selective plates. In the first panning for gp120-reactive phage, 4.6×10^{11} phage were applied to four wells and 7.7×10^5 phage were eluted. After the fourth panning 1.0×10^8 phage were eluted. From this procedure, 20 clones were selected from the Fab library for their ability to bind to glycosylated recombinant gp120 from the IIIB strain of HIV-1. Five clones were selected from the Fab library specific for binding to gp41. The panned phage surface libraries were then converted into ones expressing soluble Fab fragments for further screening by ELISA as described below.

In addition to panning on gp120 of strain IIIB and gp41, also contemplated as antigens for panning of combinatorial libraries is recombinant gp120 (IIIB strain) produced in baculovirus and recombinant gp120 (SF2 strain) produced in Chinese Hamster Ovary cells obtained as described by Steimer et al., Science, 254:105-108 (1991). Another antigen, a synthetic cyclic peptide, $N=CH-(CH_2)_3CO[SISGPGRAFYTG]NCH_2CO-Cys-NH_2$ (SEQ ID NO 48) prepared as described by Satterthwait et al., Bulletin of the World Health Organization, 68: Suppl., 17-25 (1990) corresponding to the central most conserved part of the V3 loop of gp120 was coupled to maleimide-activated BSA. The library was panned using 1, 2 or 4 ELISA wells coated with 1 μ g of protein antigen or 10 μ g BSA-peptide per well. Four rounds of panning were carried out for

each antigen as described above. Eluted phage from the final round were used to infect XL1-Blue cells. Four rounds of panning against the four antigens produced an amplification in eluted phage of between 100 and 1000 fold. The panned phage surface libraries were then converted into ones expressing soluble Fab fragments for further screening by ELISA as described below.

6) Preparation of Soluble Heterodimers and Characterization of Binding Specificity to HIV-1 Antigens

In order to further characterize the specificity of the mutagenized heterodimers expressed on the surface of phage as described above, soluble Fab heterodimers from acid eluted phage were prepared and analyzed in ELISA assays on HIV-1 derived antigen-coated plates and by competitive ELISA.

To prepare soluble heterodimers, phagemid DNA from the 20 gp120 positive clones and the 5 gp41 positive clones prepared above was isolated and digested with Spe I and Nhe I. Digestion with these enzymes produced compatible cohesive ends. The 4.7 kb DNA fragment lacking the gene III portion was gel-purified (0.6% agarose) and self-ligated. Transformation of *E. coli* XL1-Blue afforded the isolation of recombinants lacking the cpIII fragment. Clones were examined for removal of the cpIII fragment by Xho I - Xba I digestion, which should yield an 1.6-kb fragment. Clones were grown in 100 ml SB containing 50 µg/ml carbenicillin and 20 mM MgCl₂ at 37°C until an OD₆₀₀ of 0.2 was achieved. IPTG (1 mM) was added and the culture grown overnight at 30°C (growth at 37°C provides only a light reduction in heterodimer yield). Cells were pelleted by centrifugation at 4000 rpm for 15 minutes in a JA10 rotor at 4°C.

Cells were resuspended in 4 ml PBS containing 34 μ g/ml phenylmethylsulfonyl fluoride (PMSF) and lysed by sonication on ice (2-4 minutes at 50% duty). Debris was pelleted by centrifugation at 14,000 rpm in a JA20 rotor at 4°C for 15 minutes. The supernatant was used directly for ELISA analysis as described below and was stored at -20°C. For the study of a large number of clones, 10 ml cultures provided sufficient heterodimer for analysis. In this case, sonications were performed in 2 ml of buffer.

Assays as described above were also performed for the gp41-specific clones.

15 a) Screening by ELISA

The soluble heterodimers prepared above were assayed by ELISA. For this assay, gp120 and gp41 were separately admixed to individual wells of a microtiter plate as described above for the panning procedure and maintained at 4°C overnight to allow the protein solution to adhere to the walls of the well. After the maintenance period, the wells were washed five times with water and thereafter maintained for one hour at 37°C with 100 μ l solution of 1% BSA diluted in PBS to block nonspecific sites on the wells. Afterwards, the plates were inverted and shaken to remove the BSA solution. Twenty-five μ l of soluble heterodimers prepared above reactive with the specific glycoprotein substrate were then admixed to each well and maintained at 37°C for one hour to form immunoreaction products. Following the maintenance period, the wells were washed ten times with water to remove unbound soluble antibody and then maintained with a 25 μ l of a 1:1000 dilution of secondary goat anti-human IgG F(ab')₂ conjugated to alkaline phosphatase diluted in PBS containing 1% BSA. The wells were maintained at 37°C for one

hour after which the wells were washed ten times with water followed by development with 50 μ l of p-nitrophenyl phosphate (PNPP). Color development was monitored at 405 nm. Positive clones gave A405 values of >1 (mostly >1.5) after 10 minutes, whereas negative clones gave values of 0.1 to 0.2.

Approximate concentrations of gp120-reactive Fab were determined by ELISA using a sandwich ELISA as described by Zebedee et al., Proc. Natl. Acad. Sci., USA, 89:3175-3179 (1992) and are presented in the first column of Figure 6. In addition, since Fabs are expressed in E. coli and the fraction of correctly assemble protein can vary, the amount of Fab reacting with gp120 was also assessed by ELISA titration. That data is also presented in Figure 6 in the second column.

For the clones panned against the HIV-1 derived antigens, after conversion of the panned phage surface libraries to ones expressing soluble Fab fragments, 30-40 colonies were used to transform XL1-Blue cells and the supernates screened in ELISA assays against the antigen used in panning. Generally greater than 80% of the supernates tested positive. A representative number of positives were then selected from each antigen panning for further analysis.

(b) Competitive ELISA with Soluble gp120 and CD4

Immunoreactive heterodimers as determined in the above ELISA were then analyzed by competition ELISA to determine the affinity of the selected heterodimers. The ELISA was performed as described above on microtiter wells separately coated with 5 μ g/ml of gp120 or soluble CD4 (American Biotechnologies) in 0.1 M bicarbonate buffer at pH 8.6. Increasing concentrations of soluble or free gp120 ranging in concentration from

10⁻¹¹ M up to 10⁻⁷ M diluted in 0.5% BSA/0.025% Tween
20/PBS were admixed with soluble heterodimers, the
dilutions of which were determined in titration
experiments that resulted in substantial reduction
of OD values after a 2-fold dilution. For the CD4
competition assays, increasing concentrations of
soluble or free CD4 ranging in concentration from
10⁻¹¹ M up to 10⁻⁶ M diluted in 0.5% BSA/0.025% Tween
20/PBS were admixed with soluble heterodimers. The
plates were maintained for 90-120 minutes at 37°C
and carefully washed ten times with 0.05% Tween
20/PBS before admixture of alkaline
phosphatase-labelled goat anti-human IgG F(ab')₂ at
a dilution of 1:500 followed by maintenance for 1
hour at 37°C. Development was performed as
described for ELISA.

To establish the relationship between
neutralizing ability as described in Example 3
below could be related to antigen binding affinity
of HIV-1-specific Fabs, competition ELISAs were
carried out where soluble gp120 was competed with
gp120 coated on ELISA plates for Fab binding.
Figure 7 shows that all Fabs were competed from
binding to gp120 with a IC₅₀ of approximately 10⁻⁹ M
free gp120. In addition as shown in Example 3,
there is no correlation between antigen affinity
and neutralization. The Fabs tested included Fabs
4, 12, 21 and 7 that are members of the same groups
as determined by sequence analysis and comparison
as described in Example 9. Fabs 13, 27, 6, 29, 2
and 3 are all members of the different groups as
determined by sequence analysis and comparison as
described in Example 9. Loop 2 is an Fab fragment
selected from the same library as the other Fabs
but which recognizes the V3 loop. Only with the V3
loop peptide was competition carried out with gp120
from the SF2 strain.

To investigate whether neutralization could be associated with blocking of the gp120-CD4 interaction, competition ELISAs were carried out with soluble CD4 competing with Fabs for binding to gp120-coated ELISA wells. The results are shown in Figure 8. P4D10 and loop 2 are controls not expected to be competed by CD4. P4D10 is a mouse monoclonal antibody reacting with the V3 loop of gp120 (IIIB). Loop 2 Fab competition was carried out using gp120 (SF2). As shown in Figure 8 the binding of all Fabs with the exception of the controls was inhibited with an IC_{50} of approximately 10^{-8} M of soluble CD4. In addition, no difference was detected between the neutralizing and non-neutralizing Fabs to gp120 inhibited by CD4. This implies that blocking of the CD4-gp120 interaction is unlikely to be an important factor in Fab neutralization of the HIV-1 virus.

Similar competition assays were performed with the Fabs panned against the four HIV-1 derived antigens. The 19 Fabs derived from panning against gp120 (IIIB) showed apparent affinities (1/concentration at 50% inhibition) for gp120 (IIIB) in the range 10^7 - 10^9 M with most being $1-3 \times 10^{-8}$ M. The panning procedure tends to select strongly for tight binders so a grouping into a relatively narrow band of affinities was expected. Of 16 Fabs derived from panning against gp160 (IIIB), 6 were also reactive with gp120 (IIIB) and competition ELISAs showed they had similar apparent affinities as the gp120-panned Fabs. The non-gp120 reactive clones from the gp160 panning showed a lower ELISA reactivity with gp160 and could not be satisfactorily competed with gp160. They may be directed against gp41 but were not pursued here. Eight Fabs derived from panning against gp120 (SF2) also showed strong ELISA reactivity with gp120 (IIIB) and gave similar apparent binding

5 affinities. Four Fabs were derived from panning against the V3 loop peptide. Of these Fabs, 2 reacted in ELISA with gp120 (SF2) but none with gp120 (IIIB). The apparent binding affinity of these loop binders to gp120 (SF2) was 10^{-8} M.

10 To complete the survey in terms of strain cross-reactivity of Fabs, those derived from the gp120 and gp160 (IIIB) pannings were examined for ELISA reactivity with gp120 (SF2). All were reactive. Therefore, all the Fabs examined, with the exception of those selected by panning against the V3 loop peptide, bound to gp120 from IIIB and SF2 strains.

15 The Fabs were screened for CD4 inhibition of their binding to gp120 (IIIB) immobilized on ELISA wells. All, again with the exception of the V3 loop binders, showed sensitivity to CD4 inhibition. The inhibition constants were in the range 10^{-7} to 10^{-9} M.

20

(c) Binding Affinity Determination
Using Surface Plasmon Resonance

Binding affinities were determined for six of the Fabs using surface plasmon resonance. Surface plasmon resonance was performed as it is a more accurate method for measuring affinity than competition ELISA. The six Fabs were chosen based upon sequence analysis which revealed that the heavy chains could be organized into 7 groups (Example 9). Each group contained members with identical V-D and D-J joining regions, implying a common clonal origin with varying numbers of differences elsewhere in the VH domain. Six Fabs were chosen as a representative of each respective group for further study as described herein. The single member of the seventh group was not included in these studies. The binding affinities of the six Fabs that are directed against the CD4 binding site of the gp120 envelope

25

30

35

glycoprotein were determined using surface plasmon resonance as follows.

A Pharmacia BIAcore machine was used for the binding affinity determinations as previously described in Malmberg, et al., J. Immunol., 35:643-650 (1992) and Mattsson, et al., J. Immunol. Meth., 145:229-240 (1991). Optimization for the Fab fragments involved a number of steps. Two separate channels on a biosensor chip were coated with gp120 derived from the HIV-1 strain LAI (Repligen, Cambridge MA) such that one channel could be used for the determination of on-rate constants (k_{on}) and the other for the determination of off-rate constants (k_{off}).

For immobilization of antigen on the sensor surfaces, a flow rate of 5 μ l/min of PBS, pH 7.4 was established over the biosensor chip. The chip was then activated by injecting 30 μ l of activation solution (Pharmacia Biosensor, 50% 0.2 M N-ethyl-N'-(3-diethylaminopropyl)-carbodiimide, 50% N-hydroxysuccinimide). The flow rate was then adjusted to 10 μ l/min and the gp120 was injected in 10 mM sodium acetate buffer, pH 4.5. When association rates were to be determined, 25 μ l of gp120 at 10 μ g/ml was injected (a final level of 4000 Response Units (RU)). Twenty μ l of gp120 at 2 μ g/ml were injected for the determination of dissociation constants (a final level of 800 RU). In both cases, a flow rate of 5 μ l/min was reestablished following the gp120 injection and the chip was blocked from any further immobilization by the injection of 30 μ l of 1 M ethanolamine, pH 8.5 (Pharmacia Biosensor).

For determination of on-rate constants (k_{on}), a series of dilutions were made for each Fab to give

5 final concentrations in the range of 1 to 20 $\mu\text{g/ml}$.
30 μl of each Fab solution was injected in separate
experiments over the immobilized gp120 at a flow
rate of 5 $\mu\text{l/min}$. The change in response per unit
time (dR/dt) was plotted against time (t) for each
concentration. The slopes of each of these graphs
were then plotted against their corresponding
concentrations to give a final graph from which the
on-rate constant could be read.

10 For determination of off-rate constants (k_{off}),
30 μl of each Fab solution at 150 $\mu\text{g/ml}$ were
injected over the immobilized antigen at a flow
rate of 5 $\mu\text{l/min}$. Once the reaction had reached
equilibrium, the Fab was removed from the antigen
15 at a constant flow rate of 50 $\mu\text{l/min}$. A plot was
then made of $\ln(R_1/R_0)$ against $t_1 - t_0$ for the
dissociation phase. R_1 is the response at time t_1
and R_0 is the initial response at time t_0 . The
slope of this graph was taken to be the off-rate
20 constant. Affinities (K_a) were then calculated and
expressed as $k_{\text{on}}/k_{\text{off}}$.

The apparent affinities of the panel of
recombinant Fabs isolated from the donor as
determined in competition ELISA and surface plasmon
25 resonance were compared. Values of approximately
 10^8M^{-1} were obtained by competition ELISA as
described in Example 2b6c in which the soluble and
immobilized gp120 competed for binding to Fab in
bacterial supernatants. Such a methodology only
30 gives an approximate measure of affinity.
Therefore, the affinities of six of these Fabs were
measured using real-time biospecific interaction
analysis (surface plasmon resonance) in order to
obtain more accurate affinity constant values. The
35 results are reproducible with a standard deviation
from the mean of approximately 5% as determined by
calculating a number of the affinity constants in
triplicate. All Fabs examined have affinities in

the range of 5×10^7 to $1 \times 10^8 \text{ M}^{-1}$ as determined in surface plasmon resonance (Table 4). These values are in broad agreement with those derived from competition ELISA. These values imply no correlation between affinity for recombinant gp120 derived from LAI and the ability to neutralize the HXBc2 clone of HIV-1 derived from LAI as assessed in Example 3c.

Table 4

Fab	$k_{\text{on}} (\text{M}^{-1} \text{s}^{-1})$	$k_{\text{off}} (\text{s}^{-1})$	$K_{\text{a(M-1)}}$
b3	9.6×10^3	1.8×10^{-4}	5.1×10^7
b6	1.6×10^4	1.6×10^{-4}	9.7×10^7
b11	5.6×10^4	4.3×10^{-4}	1.3×10^8
b12	4.5×10^4	4.3×10^{-4}	1.1×10^8
b13	1.1×10^4	1.4×10^{-4}	7.9×10^7
b14	6.0×10^4	6.5×10^{-4}	9.2×10^7

Also contemplated are competition ELISA and surface plasmon resonance assays where the binding of HIV-1 recombinant Fabs of this invention is performed in the presence of excess Fabs of this invention as well as those HIV-1 antibodies, polyclonal or monoclonal, present in patient sera, either asymptomatic or symptomatic, or obtained by other means such as EBV transformation and the like. The ability of an exogenously admixed antibody to compete for the binding of a characterized Fab of this invention will allow for the determination of equivalent antibodies in addition to unique epitopes and binding specificities.

3. Neutralizing Activity of Recombinant Human Fab
Fragments Against HIV-1 In Vitro

5 Binding of antibodies to viruses can result in
loss of infectivity or neutralization and, although
not the only defense mechanism against viruses, it
is widely accepted that antibodies have an
important role to play. However, understanding of
the molecular principles underlying antibody
10 neutralization is limited and lags behind that of
the other effector functions of antibody. Such
understanding is required for the rational design
of vaccines and for the most effective use of
passive antibody for prophylaxis or therapy. This
15 is particularly urgent for the human
immunodeficiency viruses.

A number of studies have led to the general
conclusion that viruses are neutralized by more
than one mechanism and the one employed will depend
20 on factors such as the nature of the virus, the
epitope recognized, the isotype of the antibody,
the cell receptor used for viral entry and the
virus:antibody ratio. The principle mechanisms of
neutralization can be considered as aggregation of
25 virions, inhibition of attachment of virus to cell
receptor and inhibition of events following
attachment such as fusion of viral and cellular
membranes and secondary uncoating of the virion.
One of the important features of the third
30 mechanism is that it may require far less than the
approximately stoichiometric amounts of antibody
expected for the first two mechanisms since
occupation of a small number of critical sites on
the virion may be sufficient for neutralization.
35 For instance it has been shown that neutralization
of the influenza A virion obeys single hit kinetics
as described by Outlaw et al., Epidemiol. Infect.,
106:205-220 (1992).

Intensive studies have been carried out on antibody neutralization of HIV-1. For review, see Nara et al., FASEB J., 5:2437-2455 (1991). Most have focussed on a single linear epitope in the third hypervariable domain of the viral envelope glycoprotein gp120 known as the V3 loop. Antibodies to this loop are suggested to neutralize by inhibiting fusion of viral and cell membranes. Binding to the loop resulting in neutralization can occur prior to virus-cell interaction or following gp120 binding to CD4. See, Nara, In *Retroviruses of Human Aids and Related Animal Diseases*, eds. Girard et al., pp. 138-150 (1988); Linsely et al., J. Virol., 62:3695-3702 (1988); and Skinner et al., J. Virol., 67:4195-4200 (1988). Features of the V3 loop are sequence variability within the loop [Goudsmit et al., FASEB J., 5:2427-2436 (1991) and Albert et al., AIDS, 4:107-112 (1990)] and sensitivity of neutralizing antibodies against the loop to sequence variations outside the loop [Nara et al., FASEB J., 5:2437-2455 (1991); Albert et al., supra; McKeating et al., AIDS, 3:777-784 (1989); and Wahlberg et al., AIDS Res. Hum. Retroviruses, 7:983-990 (1991). Hence anti-V3 loop antibodies are often strain specific and mutations in the loop in vivo may provide a mechanism for viral escape from antibody neutralization.

Recently considerable interest has focused on antibodies capable of blocking CD4 binding to gp120. A number of groups have described the features of these antibodies as (a) reacting with conformational i.e., non-linear epitopes, (b) reacting with a wide range of virus isolates and (c) being the predominant neutralizing antibodies in humans after longer periods of infection. See, Berkower, et al., J. Virol., 65:5983-5990 (1991); Steimer et al., Science, 254:105-108 (1991); Ho et al., J. Virol., 65:489-493 (1991); Kang et al.,

Proc. Natl. Acad. Sci., USA, 88:6171-6175 (1991);
Posner et al., J. Immunol., 146:4325-4332 (1991);
and Tilley et al., Res. Virol., 142:247-259 (1991).

5 Neutralizing antibodies of this type would appear
to present a promising target for potential
therapeutics. The mechanism(s) of neutralization
of these antibodies is unknown although there is
some indication that this may not be blocking of
10 virus attachment since a number of mouse monoclonal
antibodies inhibiting CD4 binding to gp120 are
either non-neutralizing or only weakly
neutralizing.

The generation of human monoclonal antibodies
against the envelope of HIV-1 as described by
15 Burton et al., Proc. Natl. Acad. Sci., USA,
88:10134-10137 (1991) using combinatorial libraries
allows a novel approach to the problem of
neutralization. Given the lack of a
20 three-dimensional structure for gp120 and the
complexity of the virus, the approach seeks to
explore neutralization at the molecular level
through the behavior of related antibodies. This
is possible for the following reasons: (1) the
combinatorial approach allows the rapid generation
25 of large numbers of human antibodies; (2) the
antibodies (Fab fragments) are expressed in E.coli
and can readily be sequenced; and (3) antibodies
have similar sequences and common structural motifs
allowing functional differences to be meaningfully
30 correlated with primary structure.

Neutralization studies were performed as
described herein on the human recombinant Fab
fragments from 20 clones against gp120 prepared as
described in Examples 1 and 2, all of which are
35 strain cross-reactive and inhibited by CD4 from
binding to gp120. The results presented herein
show that neutralization was not effected by virus
aggregation or cross-linking of gp120 molecules on

the virion surface and was not correlated with blocking of the interaction between soluble CD4 and recombinant gp120.

Neutralization studies were also performed as described herein on the human recombinant Fab fragments from the gp41-reactive clones prepared as described in Examples 1 and 2. The results are presented below.

Two different assays, a p24 ELISA assay and a syncytium assay, were performed to measure neutralization ability of the recombinant human HIV-1 immunoreactive Fabs. An additional assay, a plaque assay, was performed for determining the neutralization effectiveness of the gp41-reactive Fabs. In plaque assays, CD4+ cells were cultured in the presence or absence of soluble gp41-reactive Fabs prior to inoculation with virus. Inhibition of infectivity, also referred to as neutralization, by antibodies was expressed as the percent of plaque formation in the cultures compared to cells exposed to PBS alone.

Neutralization assays were also performed with an antibody molecule consisting of the light chain and the VH region of the Fab 12 and the constant regions (CH1, CH2, and CH3) of an IgG1 molecule. Quantitative infectivity microplaque and syncytial formation assays to measure neutralization were performed with the b12 IgG1 and laboratory isolates MN and IIIB of HIV-1 virus. In the syncytial formation assay, virus was grown in H9 cells and infectivity measured by culturing monolayers of CEM-SS target cells with 100-200 syncytial forming units (SFUs) of virus, in the presence or absence of antibody. p24 ELISA and microplaque formation assays were also performed with primary clinical isolates of the HIV-1 virus.

In addition, the ability of the recombinant human HIV-1 immunoreactive Fabs b3, b6, b12, b13,

and b12 to neutralize the HXBc2 molecular clone of gp120 derived from HTLV-IIIB (LAI) was determined in an envelope complementation assay. The supernatant containing recombinant HIV-1 virions from cotransfected COS-1 cells was incubated with the recombinant Fabs prior to incubation with Jurkat cells. The recombinant HIV-1 virions contained the HXBc2 clone of HIV-1 strain LAI which encodes a chloramphenicol acetyltransferase (CAT) gene. Upon infection of Jurkat cells with the recombinant HIV-1 virions, the CAT gene was expressed and CAT activity measured. Activity of the CAT gene was therefore an indication of infectivity of the Jurkat cells with the recombinant HIV-1 virion. Lack of CAT activity indicated the Jurkat cells were not infected with the recombinant HIV-1 virion.

For some of these assays, the recombinant Fabs were first purified. One liter cultures of SB containing 50 µg/ml carbenicillin and 20 mM MgCl₂ were inoculated with appropriate clones and induced 7 hours later with 2 mM IPTG and grown overnight at 30°C. The cell pellets were sonicated and the resultant supernatant were concentrated to a 50 ml volume. The filtered supernatants were loaded on a 25 ml protein G-anti-Fab column, washed with 120 ml buffer at a rate of 3 ml/minute and eluted with citric acid at pH 2.3. The neutralized fractions were then concentrated and exchanged into 50 mM MES at pH 6.0 and loaded onto a 2 ml Mono-S column at a rate of 1 ml/minute. A gradient of 0-500 mM NaCl was run at 1 ml/minute with the Fab eluting in the range of 200-250 mM NaCl. After concentrating, the Fabs were positive when titered on ELISA against gp120 and gave a single band at 50 kD by 10-15% SDS-PAGE. Concentration was determined by absorbance measurement at 280nm using an extinction coefficient (1 mg/ml) of 1.4.

a. Neutralization as Measured by the p24
ELISA Assay

For this assay, diluted tissue culture supernatants of HIV-1 IIIB or MN-infected peripheral blood mononuclear cells (PBMC) (50TCID₅₀ (50% tissue culture infectious dose), 100 µl) were maintained for 2 hours at 37°C with serial dilutions (1:2), beginning at a dilution of 1:20, of recombinant Fab supernates prepared in Example 2b6). Control Fab supernates were also provided that included human neutralizing sera, a known human neutralizing monoclonal antibody 2F5 and the Fab fragment derived from that antibody by papain digestion, and a known mouse neutralizing monoclonal antibody and its F(ab')₂ fragment as described by Broliden et al., J. Virol., 64:936-940 (1990). PBMC (1 x 10⁵ cells) were admixed to the virus/antibody admixture and maintained for 1 hour at 37°C. Thereafter, the cells were washed and maintained in RPMI 1640 medium (GIBCO) supplemented with 10% fetal calf serum, 1% glutamine, antibiotics and IL-2. The culture medium was changed at days 1 and 4. At 7 days post-infection, supernates were collected and analyzed by HIV-1 p24 antigen capture ELISA as described by Sundqvist et al., J. Med. Virol., 29:170-175 (1989) the disclosure of which is hereby incorporated by reference. Neutralization was defined as positive if an 80% or greater reduction of optical density at 490nm in the culture supernatant occurred as compared to negative Fab or negative human serum. Tests with all Fabs, mAbs and sera were repeated on at least two occasions.

b. Quantitative Infectivity Assay Based on
Syncytial Formation

A quantitative neutralization assay with the MN strain of HIV-1 was performed as described

by Nara et al., AIDS Res. Human Retroviruses,
3:283-302 (1987), the disclosure of which is hereby
incorporated by reference. Monolayers of CEM-SS
target cells were cultured with virus, in the
5 presence or absence of antibody, and the number of
syncytia forming units determined 3-5 days later.
An equivalent amount of virus was used in the
assays to allow direct comparison of the various
antibody concentrations tested. The assays were
10 repeatable over a virus-surviving fraction range of
1 to 0.001 within a 2 to 4-fold difference in the
concentration of antibody ($P < 0.001$).

c. Neutralization as Measured by the
15 Envelope Complementation Assay

The ability of purified recombinant Fabs
b3, b6, b11, b12, b13, and b14 to neutralize the
HXBc2 gp120 molecular clone of the HIV-1 (LAI)
isolate was assessed in an envelope complementation
20 assay (Helseth et al., J. Virol., 65:2119-2123
(1991)). Briefly, COS-1 cells were cotransfected
with a plasmid expressing envelope glycoprotein 120
derived from HIV-1 (LAI) and a plasmid containing
an env-defective HXBc2 clone and encoding the
25 bacterial CAT gene. Equal fractions of the cell
supernatants containing recombinant virions were
incubated at 37°C for 1 hour with varying
concentrations of recombinant Fab (0.1 - 20 µg/ml)
or control monoclonal antibody 110.4 prior to
30 incubation with Jurkat cells. Three days post-
infection, the Jurkat cells were lysed and CAT
activity measured. Neutralization was expressed as
a decrease in the percentage of residual
chloramphenicol transferase (CAT) activity.
35 Control monoclonal antibody 110.4 is a strongly
neutralizing antibody directed to the V3 loop of
the HXBc2 HIV-1 strain.

d. Results of the Neutralization Assays for gp120

Assays were generally repeated at least twice with reproducible results. For the data reported in Figure 6, the gp120-specific Fab supernates were divided into two parts, one being used in the p24 assay and the other in the syncytia assay. A dash (-) indicates that there was no neutralization at 1:20 dilution in the p24 assay and 1:16 in the syncytial assay (with most clones showing no detectable neutralization at a 1:4 dilution). Neutralization titers are indicated in the figure. For the p24 assay, the titer corresponds to the greatest dilution producing >80% reduction in absorbance in ELISA. For the syncytia assay, Fabs 4 and 12 produced >95% neutralization at a 1:4 dilution of supernate and 80 and 70% reduction at 1:128 dilution respectively. These Fabs were effective neutralizers in both types of assays. They have also been shown to neutralize infection by IIIB and RF strains using a PCR-based assay of proviral integration. Fabs 6 and 7 showed no neutralization in the syncytia assay but other supernate preparations showed activity. Fab 13 was consistently effective in the p24 assay but not in the syncytia assay. A number of other clones show lower levels of neutralizing ability.

Fabs were purified from a selection of some of the clones as described above and used in both neutralization assays. As shown in Figure 9, Fabs 4 and 12 were again effective in both assays at similar levels with for example 50% inhibition of syncytial formation at an Fab concentration of approximately 20 nM (1 µg/ml). The results shown are derived from the syncytia assay using the MN strain. Fabs 7 and 21 were equally effective in the syncytial assay but somewhat less so in the p24 assay. The p24 assay indicated greater than 80%

neutralization of HIV-1 MN strain for Fab 4 at 3, Fab 7 at 15, Fab 12 at 3, Fab 13 at 4 and Fab 21 at 7 $\mu\text{g/ml}$, respectively. Fab 13 however was ineffective in the syncytial assay at 25 $\mu\text{g/ml}$.

5 For the IIIB strain, greater than 80% neutralization was observed for Fab 4 at 13, Fab 7 at 15, Fab 12 at 7 and Fab 21 at 14 $\mu\text{g/ml}$, respectively. Although Fab 11 was not effective in neutralization assays when unpurified as shown in

10 Figure 6, following purification, Fab 11 was equally effective as Fab 12 in neutralizing HIV-1. For this reason, the Fab is being deposited with the ATCC as described in Example 12 along with Fab 12 and Fab 13.

15 The ability of purified recombinant Fabs b3, b6, b11, b12, b13, and b14 to neutralize the HXBc2 gp120 molecular clone of the HIV-1 (LAI) isolate was assessed in an envelope complementation assay. Figure 23 shows the concentration dependence of Fab

20 neutralization of the HXBc2 clone in this assay. All of the Fabs neutralize effectively at the highest concentration measured (20 $\mu\text{g/ml}$). Irrelevant Fabs, Fabs directed to surface glycoproteins on other viruses such as RSV, do not

25 neutralize in this assay. Examination of the lower concentrations clearly reveals that Fab b12 is the most effective neutralizer. The neutralizing potency of Fab b12 was greater than that of the 110.4 whole monoclonal antibody tested in parallel.

30 The 110.4 antibody is one of the most potent antibodies directed against the V3 loop of the HXBc2 HIV-1 strain (Thali, M. and J. Sodroski, unpublished observations). In other studies, Fab b12 has been found to show exceptional neutralizing

35 ability towards laboratory (Example 3 and Barbas et al., Proc. Natl. Acad. Sci., USA, 91, in press (1994)) and field isolates of HIV-1 as described in Example 5.

There are a number of conclusions arising from the data shown in the Figures 6, 9 and 23. It is apparent that HIV-1 can be neutralized without virion aggregation or cross-linking of gp120 molecules on the virion surface since monovalent Fab fragments are effective. To further confirm this finding, a Fab fragment was produced by papain digestion of a known neutralizing human monoclonal antibody. As shown in Figure 6, the Fab fragment was approximately equally effective as the whole IgG in neutralization of the MN strain of HIV-1. This is consistent with results on Fabs prepared from two mouse monoclonal antibodies to the V3 loop. An F(ab')₂ fragment of a mouse monoclonal antibody was somewhat less effective than the parent IgG in neutralization of the MN strain. Interestingly, the fragments from these control antibodies were relatively poor in neutralizing the IIIB strain of HIV-1. The results also show that there appears to be a difference between the two assays employed since Fab 13 was consistently effective in one assay but not the other. The principal variables were the incubation time of the virus and antibody prior to infection (2 hours for the p24 assay and 0.5 hours for the syncytial assay), the amount of virus used for infection, the cells used to propagate virus (human PBMCs for the former and H9 cells for the latter) and the cells infected (human PBMCs for the former and CEM.SS cells for the latter). Of these, there is a strong possibility that the MN virus used in the two assays, having been passaged through different cells, is critically different.

The Fabs show a spectrum of neutralizing ability for gp120 from a molecular clone HXBc2 derived from the HIV-1 strain LAI in the envelope complementation assay. Fab b12 exhibited the greatest potency of neutralization and was even

more effective in this assay than a whole antibody directed to the V3 loop of gp120. Neutralizing ability is not correlated with either the apparent affinity of the Fab for gp120 derived from the recombinant HIV-1 strain LAI as estimated by competition ELISA or the affinity for gp120 derived from HIV-1 strain LAI as determined by surface plasmon resonance. For example, Fabs b6, b12, and b14 have very similar affinities by surface plasmon resonance (Table 4) but different neutralization ability in the envelope complementation assay (Figure 23). Similarly, neutralization is not correlated with the ability of the Fab to compete with soluble CD4 in a competition ELISA.

e. Results of the Neutralization Assays for gp41

The gp41-reactive Fabs exhibited specificity to the conformation epitope of gp41 including amino acid residues in positions 565-585 and 644-663. The five selected gp41-specific Fabs were designated DL 41 19, DO 41 11, GL 41 1, MT 41 12 and SS 41 8. Neutralization assays were performed as described above for the gp120-reactive Fabs. In the plaque assays, the data shown is the concentration of Fab in micrograms/milliliter required to achieve 50% of neutralization. The data for the other two neutralization assays is also expressed in micrograms/milliliter of Fab required to neutralize infection as defined in the description of the p24 and syncytial assays above. The results of the three neutralization assays, plaque, syncytial and p24, are presented in Table 5. The MN and IIIB HIV strains were used as indicated in Table 5 for the assays. The abbreviation "ND" stands for not determined when indicated in the table.

Table 5
Assay/Strain

	Fab	<u>Plaque</u>		<u>Syncytial</u>	<u>P24</u>	
		<u>MN</u>	<u>IIIB</u>	<u>IIIB</u>	<u>MN</u>	<u>IIIB</u>
5	DL 41 19	<4	<40	1.4	ND	ND
	DO 41 11	<40	7.1	2.3	0.9	ND
	GL 41 1	<4	<4	1.7	ND	3.5
	MT 41 12	<40	<40	5.5	4.5	4.5
	SS 41 8	<4	<4	2.2	ND	7.1

10

As shown in Table 5, all five Fabs were effective at neutralizing both MN and IIIB strains of HIV in either plaque, syncytial or p24 assays. Fabs DL 41 19 and DO 41 11 exhibited strain specificity in the plaque assay where the former was ten-fold more effective at inhibiting plaque formation with the MN strain than with the IIIB strain. The opposite specificity was seen with the DO 41 11 Fab. However, both Fabs exhibited comparable neutralization as measured by the syncytial assay. Two Fabs, GL 41 1 and SS 41 8, were equally effective at inhibiting plaque formation with either MN or IIIB strains. The Fab MT 41 12 was similarly not strain-specific although neutralization required 10 fold more antibody. No strain specificity was evident when Fab MT 41 12 was used in p24 assays where the same amount of antibody was equally effective. All five antibodies were neutralized IIIB as measured in the syncytial assay.

30

Thus, the five gp41-specific Fabs neutralized HIV-1 MN and IIIB in at least two of the three assays used for measuring neutralizing activity. Moreover, strain specificity was prevalent in two of the five assays as measured by the plaque assay. Based on these differential neutralization characteristics, the gp41-specific Fabs provide useful therapeutic reagents for neutralizing HIV-1.

35

4. Construction of a Mammalian Expression Vector
pEel2 Combo BM 12 for the Expression of an
IgG1 Antibody Molecule with the Fab from b12
(b12 IgG1)

5

Although Fab b12 is capable of neutralizing some primary isolates, the corresponding whole antibody molecule is likely to be more effective. The whole antibody, consisting of the Fab fragment and the Fc domain, participates in the elimination of foreign cells by first binding specifically to the foreign cell via the Fab portion and interacting with other cells in the immune system via the Fc domain. The Fc domain also enables the antibody to bind complement.

15

Fab b12 was converted to a whole IgG1 molecule (b12 IgG1) by cassetting the variable heavy chain (VH) and light chain genes into a vector created for high-level mammalian expression. b12 IgG1 used in the neutralization studies was prepared by expression in Chinese hamster ovary (CHO) cells and purified by affinity chromatography.

20

The strategy to convert the Fab b12 to a whole IgG1 molecule was similar to that described previously for the generation of a whole antibody beginning with a phage derived Fab (Bender, et al., Hum. Antibod. Hybridomas, 4:74-79 (1992)).

25

a. Construction of b12 Heavy Chain IgG1 pSG-
5 Mammalian Expression Vector

30

1) Modification of b12 Heavy Chain
Variable Region to Introduce a Kozak
Sequence, Mammalian Leader Sequence,
and Human VH Consensus Sequence

35

First, the b12 VH region was cloned into a pSG-5 expression vector (Green et al., Nucl. Acids Res., 16:369 (1988)) to fuse the b12 VH to the heavy chain constant domains (CH1, CH2, and

CH3) of an IgG1 antibody molecule. The double-stranded Fab b12 DNA was used as a template for isolating the gene encoding the VH region of the Fab b12, the amino acid residue sequence of which is listed in SEQ ID NO 66. Fab b12 DNA and mouse B73.2 IgG1 DNA (Whittle, et al., Protein Eng., 1:499 (1987) and Bruggmeman, et al., J. Exp. Med., 166:1351 (1987)) were used as templates for a PCR amplification for the construction of a DNA fragment consisting of the unique Kozak sequence for the control of heavy chain expression, the mouse B72.3 heavy chain leader sequence (MEWSWVFLFFLSVTTGVHS (SEQ ID NO 155 from amino acid residue sequence 1 to 20)), the human VH consensus sequence (QVQLVQ (SEQ ID NO 155 from amino acid residue sequence 21 to 26)), and the VH region of the Fab b12. Altering the beginning of the VH from the mouse consensus sequence to the human consensus sequence also destroyed the original Xho I cloning site. The restriction sites EcoR I and Sst I were introduced in the amplification reaction and were located at the 5' and 3' ends of the fragment, respectively. The procedure for creating the modified VH fragment by combining the products of the two separate PCR amplifications is described below.

The primer pair, HC-1 (SEQ ID NO 157) and HC-2 (SEQ ID NO 158) as shown in Table 10, was used in the first PCR reaction to amplify a portion of the Fab b12 VH gene and incorporate the human heavy chain consensus sequence into the 5' end of the VH fragment and introduce an Sst I cloning site in the 3' end of the VH fragment. In addition, the 5' PCR primer introduces sequences into the VH fragment which form 27 base pairs of homology with the mouse leader sequence fragment prepared below. The 27 base pairs of homology in the fragments is used in a subsequent PCR reaction to fuse the two PCR

products (Yon and Fried, Nucl. Acids Res., 17:4895 (1989)) to form a modified VH fragment consisting of the EcoR I cloning site, the mouse leader sequence 72.3, the human consensus sequence, the remaining VH coding sequence, and the Sst I cloning site. For the PCR reactions, 1 μ l containing 100 ng of Fab b12 DNA was admixed with 10 μ l of 10X PCR buffer in a 0.5 ml microfuge tube. To the DNA admixture, 8 μ l of a 2.5 mM solution of dNTPs (dATP, dCTP, dGTP, dTTP) was admixed to result in a final concentration of 200 micromolar (μ M) of each dNTP. 1 μ l (equivalent to 20 picomoles (pM)) of the 5' forward HC-1 primer and 1 μ l (20 pM) of the 3' backward HC-2 primer were admixed into the DNA solution. To the admixture, 73 μ l of sterile water and 2.5 units of Taq DNA polymerase was added. Two drops of mineral oil were placed on top of the admixture and 35 rounds of PCR amplification in a thermocycler were performed. The amplification cycle consisted of 52°C for 1 minute, 72°C for 2 minutes and 94°C for 0.5 minutes.

The primer pair, HC-3 (SEQ ID NO 159) and HC-4 (SEQ ID NO 160) as shown in Table 10, was used in a separate PCR reaction to amplify the mouse B72.3 leader sequence and incorporate an EcoR I cloning site at the 5' end of the fragment and to introduce a 27 base pair sequence which has homology to the modified VH fragment prepared above. Double-stranded DNA encoding the mouse B73.2 IgG1 (Whittle, et al., supra) was used as a template for preparation of the mouse 72.3 leader sequence. The PCR reaction to prepare the mouse leader sequence fragment was performed using the same conditions as described above for the preparation of the modified VH fragment.

The resultant PCR modified b12 VH DNA fragment and mouse leader sequence fragment were purified by electrophoresis in a 2.5% Nu-Sieve agarose gel

(FMC). The area in the agarose containing the modified b12 VH DNA fragment and mouse leader sequence fragment were excised from the agarose.

5 A third PCR amplification using the primer pairs, HC-1 (SEQ ID NO 157) and HC-3 (SEQ ID NO 159) as shown in Table 10, was performed to fuse the mouse leader fragment with the modified VH fragment. The primers used for this amplification were designed to preserve an EcoR I site, a unique Kozak sequence, and the mouse B72.3 heavy chain leader sequence on the 5' end of the amplified fragment and to preserve the Sst I cloning site on the 5' end of the amplified fragment. The templates used in this PCR reaction were the two purified PCR reaction products described above. 10 The PCR reaction and subsequent purification of the PCR product were performed as described above. 15

2) Modification of b12 Heavy Chain
Variable Region to Eliminate a BglII
Restriction Site

20 The b12 modified heavy chain fragment prepared in Example 4a1 contained a Bgl II cloning site at amino acid residue 87 which would interfere with the insertion of the heavy chain fragment into the pEE6 mammalian expression vector. 25 The Bgl II restriction site was therefore eliminated in a PCR reaction using primers which destroyed the Bgl II restriction site while preserving the encoded amino acid, arginine at 30 amino acid residue 87 of the modified b12 heavy chain fragment.

35 The primer pair, HC-1 (SEQ ID NO 157) and HC-6 (SEQ ID NO 162) as shown in Table 10, was used in the first PCR reaction to preserve the 5' region of the modified b12 heavy chain fragment and destroy the Bgl II restriction site at amino acid residue 87 of the heavy chain. The HC-6 primer introduces

sequences into the VH fragment which form 32 base pairs of homology with the remaining portion of the VH fragment which will be prepared as described below. The 32 base pairs of homology in the fragments was used in a subsequent PCR reaction to fuse the two PCR products (Yon and Fried, supra) to form a modified VH fragment as described above but without the Bgl II restriction site. The PCR reaction was performed and the PCR products were purified as described in Example 4a1.

The primer pair, HC-2 (SEQ ID NO 142) and HC-5 (SEQ ID NO 145) as shown in Table 10, was used in the second PCR reaction to preserve the 3' region of the modified b12 heavy chain fragment and destroy the Bgl II restriction site. The HC-5 primer introduces sequences into the VH fragment which form 32 base pairs of homology with the remaining portion of the VH fragment which was prepared in the first PCR reaction. PCR products which have incorporated the HC-5 and HC-6 primers contain 32 base pairs of overlapping sequences which are identical. It is the annealing of the two PCR products at these 32 base pairs during the subsequent PCR reaction which fuses the two portions of the VH fragment together to recreate the entire VH fragment as described in Yon and Fried (supra).

A third PCR amplification using the primer pairs, HC-1 (SEQ ID NO 157) and HC-3 (SEQ ID NO 159) as shown in Table 10, was performed to fuse the two VH fragments in which the Bgl II restriction site had been destroyed. The primers used for this amplification were designed to preserve an EcoR I site, a unique Kozak sequence, and the mouse B72.3 heavy chain leader sequence on the 5' end of the amplified fragment and the Sst I cloning site on the 3' end of the amplified fragment. The templates used in this PCR reaction

were the two purified PCR reaction products described above. The PCR reaction and subsequent purification of the PCR product were performed as described in Example 4a1.

5

Table 10

SEQ						
ID NO	Primer					
(141) ¹	HC-1	(F)	5'	CAGGTT	CAGCTGGTTCAGTCCGGGG	CT 3'
10	(142) ²	HC-2	(B)	5'	CCTTGGAGCTCACGATGACCGTGGT	TCCTTGGCCCCAGACGTCC3'
	(143) ³	HC-3	(F)	5'	GGCCGCGAATTCGCCGCCACCATGG	AATGGAGCTGGGTCTTTCTCTTCTT
					CCTGTCAGTA	3'
	(144) ²	HC-4	(B)	5'	AGCCCCGGACTGAACCAGCTGAAC	CTG 3'
15	(145) ⁴	HC-5	(F)	5'	GGAGTTGAGGAGCCTCAGGTCTGCA	GACACGG 3'
	(146) ⁴	HC-6	(B)	5'	CCGTGTCTGCAGACCTGTGGCTCCT	CAACTCC 3'
	(147)	LC-1	(F)	5'	GATGCCAGATGTGAGATCGTTCTCA	CGCAGTCT 3'
	(148) ^{3,5}	LC-2	(B)	5'	GCGGGATCCGAATTCCTAGAAATTA	AACTCTCCCCCTGTTGAAGCTCTTT
					GTGACGGGCGAACTCAG	3'
	(149) ³	LC-3	(F)	5'	GCGCGAATTCACCATGGGTGTGCCC	ACTCAGGTCCTGGGGTTGCTGCTGC
						3'
	(150)	LC-4	(B)	5'	AGACTGCGTGAGAACGATCTCACAT	CTGGCATC 3'

(151)⁶ LC-5 (F) 5' GCGCAAGCTTACCATGGGTGTGCCC
ACTCAGGTCCTGGGGTTGCTGCTGC
3'

F Forward Primer

5 B Backward Primer

¹ the Sst I cloning site is single underlined

² the primers, HC-2 and HC-4 contain
complementary sequences

³ the EcoR I cloning site is single underlined

10 ⁴ in HC-4, the G that is double underlined was
altered from an A to eliminate a Bgl II
restriction site; in HC-5, the C that is
doubleunderlined was altered from a T to
eliminate a Bgl II restriction site

15 ⁵ the base A that is double underlined was
introduced in the PCR primer to alter the
encoded amino acid from an arginine, R, to a
serine, S

20 ⁶ the HindIII cloning site is single underlined

3) Insertion of Modified b12 Heavy
Chain Variable Region into the pSG-5
Mammalian Expression Vector

The modified b12 heavy chain
25 variable region PCR product was ligated into a
mammalian expression vector (Adair, et al., Hum.
Antibod. Hybridomas, in press). The mammalian
expression vector consisted of the pSG-5 vector
(Figure 24) with a human IgG1 gene inserted at the
30 EcoR I site. The human IgG1 gene contained a VH
insert in the same reading frame as the constant
regions of the human IgG1 gene. The VH insert was
removed by digestion with EcoR I and Sst I enzymes.
The constant regions (CH1, CH2, and CH3) remained
35 in the pSG-5 vector. Transcription of the heavy

chain gene in the pSG-5 expression vector is under the control of the SV40 early promoter.

Transcriptional termination is signaled by the SV40 polyadenylation signal sequence downstream of the heavy chain sequence. The M13 intergenic region allows for the production of single-stranded DNA for nucleotide sequence determination.

The modified b12 heavy chain variable region PCR product was digested with EcoR I and Sst I and purified on a 2.5% Nu-Sieve agarose gel (FMC). The mammalian expression vector DNA containing the IgG1 sequences was digested in parallel with EcoR I and Sst I enzymes to remove the original VH region. The PCR modified heavy chain variable region was ligated to the constant regions in the mammalian expression vector using T4 DNA ligase under conditions well known to those of skill in the art and transformed into DH5 α competent cells following the manufacturer's recommended procedures (GIBCO, BRL Life Technologies, Gaithersburg, MD). The PCR modified heavy chain variable region was inserted in the same reading frame as the constant regions of the human IgG1 gene in the pSG-5 vector. Miniprep DNAs were analyzed and large scale plasmid preparations performed. The nucleotide sequence of the 5' untranslated region including the Kozak sequence, mouse B72.3 heavy chain leader sequence, heavy chain variable region, heavy chain constant regions, and SV40 signal sequence was determined by the dideoxy-nucleotide chain termination method (Sanger et al., supra).

b. Construction of a b12 Light Chain pSG-5 Mammalian Expression Vector

- 1) Modification of b12 Light Chain to Introduce a Kozak Sequence, Mammalian Leader Sequence, and Human Light Chain Consensus Sequence

The b12 light chain was cloned into a separate pSG-5 expression vector (Green et al., supra). The double-stranded Fab b12 DNA was used as a template for isolating the gene encoding the light chain of the Fab b12, the amino acid residue sequence the light chain of Fab b12 is listed in SEQ ID NO 97. Mouse B73.2 IgG1 DNA (Whittle, et al., Protein Eng., 1:499 (1987) and Bruggmeman, et al., J. Exp. Med., 166:1351 (1987)) was used as a template for isolating the mouse B73.2 leader sequence. Fab b12 and mouse B73.2 IgG1 DNA were thus used as templates for a PCR amplification for the construction of a DNA fragment consisting of the unique Kozak sequence for control of light chain expression, the mouse B72.3 light chain leader sequence (MGVPTQLGLLLWLTDARC (SEQ ID NO 153 from amino acid residue sequence 1 to 20)), and the b12 light chain beginning with a human light chain amino acid consensus sequence (EIVLTQSP (SEQ ID NO 153 from amino acid residue sequence 21 to 28)). Altering the beginning of the light chain from the mouse amino acid consensus sequence to the human amino acid consensus sequence also destroys the original Sac I cloning site. The restriction site, EcoR I, was introduced in the amplification reactions and was located at both the 5' and 3' ends of the fragment. The procedure for creating this fragment by combining the products of two separate PCR amplifications is described below.

The primer pair, LC-1 (SEQ ID NO 163) and LC-2 (SEQ ID NO 164), was used in the first PCR reaction as performed above to amplify the Fab b12 light chain gene and incorporate the human light chain consensus sequence into the fragment and the EcoR I cloning site into the 3' end of the b12 light chain fragment. For the PCR reaction, 1 μ l containing 100 ng of Fab b12 DNA was admixed with 10 μ l of 10X PCR buffer in a 0.5 ml microfuge tube. To the DNA

admixture, 8 μ l of a 2.5 mM solution of dNTPs (dATP, dCTP, dGTP, dTTP) was admixed to result in a final concentration of 200 μ M of each dNTP. 1 μ l (equivalent to 20 pM) of the LC-1 primer and 1 μ l (20 pM) of the 3' backward LC-2 primer was admixed into the DNA solution. To the admixture, 73 μ l of sterile water and 2.5 units of Taq DNA polymerase was added. Two drops of mineral oil were placed on top of the admixture and 35 rounds of PCR amplification in a thermocycler were performed. The amplification cycle consisted of 52°C for 1 minute, 72°C for 2 minutes and 94°C for 0.5 minutes.

The primer pair, LC-3 (SEQ ID NO 165) and LC-4 (SEQ ID NO 166) as shown in Table 10, was used in a separate PCR reaction to amplify the mouse light chain B72.3 leader sequence and incorporate an EcoR I cloning site at the 5' end of the fragment and to introduce a 27 base pair sequence which has homology to the modified light chain fragment prepared above. Double-stranded DNA encoding the mouse B73.2 IgG1 (Whittle, et al., *supra*) was used as a template for preparation of the mouse 72.3 leader sequence. The PCR reaction to prepare the mouse leader sequence fragment was performed using the same conditions as described in Example 4a for the preparation of the modified VH fragment.

The resultant PCR modified b12 light chain DNA fragment and light chain mouse leader sequence fragment were purified by electrophoresis in a 2.5% Nu-Sieve agarose gel (FMC). The area in the agarose containing the modified b12 light chain DNA fragment and light chain mouse leader sequence fragment were excised from the agarose.

A third PCR amplification using the primer pairs, LC-1 (SEQ ID NO 157) and LC-4 (SEQ ID NO 166) as shown in Table 10, was performed to fuse the light chain mouse leader fragment with the

modified light chain fragment. The primers used for this amplification were designed to preserve an EcoR I site, a unique Kozak sequence, and the mouse B72.3 light chain leader sequence on the 5' end of the amplified fragment and to preserve the EcoR I cloning site on the 5' end of the amplified fragment. The templates used in this PCR reaction were the two purified PCR reaction products described above. The PCR reaction and subsequent purification of the PCR product were performed as described in Example 4a1.

2) Insertion of Modified b12 Light Chain into pSG-5 Mammalian Expression Vector

The modified b12 light chain PCR product was ligated to a pSG-5 vector (Figure 24). The pSG-5 vector had the same features described in Example 4a2 but did not contain a human IgG1 gene. The modified b12 light chain PCR product was digested with EcoR I and purified on a 2.5% Nu-Sieve agarose gel (FMC). The pSG-5 vector DNA was digested in parallel with EcoR I enzyme. The PCR modified light chain was ligated to the pSG-5 vector using T4 DNA ligase (New England Biolabs, Beverly, MA) and transformed into DH5 α competent cells (GIBCO, BRL Life Technologies, Gaithersburg, MD) following manufacturer's instructions. Miniprep DNAs were analyzed and isolation of plasmid DNA performed. The nucleotide sequence of the light chain gene was determined using the dideoxy-nucleotide chain termination method (Sanger et al., supra). The nucleotide sequence of the 5' untranslated region, mouse B72.3 light chain leader sequence, light chain variable region, light chain constant region, and SV40 signal sequence was obtained. The nucleotide and amino acid residue sequences are illustrated in Figures 25A and 25B

and are given in the sequence listing as SEQ ID NOS 152 and 153.

c. Transient Expression of b12 Heavy and Light Chain Genes in pSG-5 Vectors in COS-7 Cells

1) Transient Expression of b12 IgG1 in COS-7 Cells

The human heavy and light chains in the separate pSG-5 expression vectors were cotransformed and transiently expressed in COS-7 cells. COS-7 cells (SV40 transformed African Green Monkey Kidney Cells) provide a rapid and convenient method to test the expression and function of the antibody genes. The COS-7 cells constitutively express the SV40 large T antigen which supports the transient replication of episomes carrying the SV40 origin of replication. The pSG-5 expression vector has an SV40 origin of replication. Upon transfection into COS-7 cells, the expression vectors are replicated in the nucleus to a high copy number, resulting in relatively high transient expression levels.

COS-7 cells were obtained from the American Type Culture Collection (CRL 1651) and cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal bovine serum (GIBCO BRL, Gaithersburg, MD) and 1% penicillin, and 1% streptomycin. Transfections were performed with 10 μ g of plasmid DNA per 100 mm tissue culture plate containing 1×10^6 cells. The control plate was transfected with plasmid vector DNA without an insert. The plates were incubated at 37°C after transfection. The supernatants were harvested at 48 hours and tested for gp120 binding specificity in an ELISA assay.

2) ELISA Assay for the Detection
of Binding of b12 IgG1 to gp120

Supernatants from COS-7

5 transformants were tested for binding to gp120 in
an ELISA assay. Briefly, the ELISA plate was
coated with recombinant IIIB gp120 antigen at a
concentration of 1 µg/ml. The serially diluted
supernatant containing the b12 antibody was added
to the wells and incubated at 37°C for 1 hour.
10 After washing the plate to remove unbound antibody,
a goat anti-human Ig Fc horse radish peroxidase
(HRP) conjugated secondary antibody was added and
incubated for an additional hour. An OPD substrate
for the HRP conjugated antibody was added and the
15 HRP activity detected by determining the absorbance
at 490 nm.

d. Insertion of the b12 Heavy Chain IgG1
into the pEE6 Mammalian Expression Vector
to Create pEe6HC BM 12

20 After confirmation that the antibody
molecule expressed by the heavy and light chain
pSG-5 expression vectors bound gp120 as described
in Example 4c, the heavy chain was removed from the
25 pSG-5 vector and ligated into the pEE6 mammalian
expression vector (Bebbington et al.,
Bio/Technology, 10:169 (1992)). The pEE6 vector
(Celltech, England) contains an HCMV promoter and
the glutamine synthetase gene (GS). The pEE6
30 vector was chosen because of the GS gene which
serves as a selectable marker. CHO cells are
devoid of GS activity and thus are dependent on a
supply of glutamine in the culture medium. Cells
transfected with the pEE6 vector containing the GS
35 gene are able to synthesize glutamine from
glutamate and can survive in the absence of
glutamine in the culture medium. For CHO cells,
the addition of methyl sulfoxamine (MSX) leads to

amplification of the transfected plasmid DNA.

The heavy chain pSG-5 vector was digested with EcoR I and Bgl II to remove the 5' untranslated region including the unique Kozak sequence, mouse heavy chain B72.3 leader sequence, and heavy chain variable and constant regions from the pSG-5 vector. The pEE6 vector was also digested with EcoR I and BamH I. Both the vector and heavy chain DNAs were analyzed on a 0.7% low melting point agarose (LMPA) gel. The 3.5 kb heavy chain band and the 4.68 kb pEE6 vector band were excised from the gel and ligated together in the presence of the LMPA at 15°C overnight with 1 μ l of T4 DNA ligase and 1 μ l of 10X ligase buffer (New England Biolabs, Beverly, MA). Upon ligation, the EcoR I site is reconstituted but the BamH I and BglII sites are destroyed. Prior to transformation, 5 μ l of the ligated DNA in LMPA was diluted with 20 μ l of TCM buffer (10 mM tris, 10 mM CaCl₂, and 10 mM MgCl₂). Only 10 μ l of the 25 μ l was used for the transformation. The ligated circular plasmid DNA construct was transformed into maximum efficiency DH5 α competent cells. The standard protocol for transformation was used, wherein the DNA and 100 μ l of the competent bacterial mix (GIBCO BRL, Gaithersburg, MA) were incubated on ice for 20 minutes and heat shocked at 42°C followed by incubation on ice for 2 minutes. About 900 μ l of SOC (GIBCO BRL, Gaithersburg, MA) was added to the transformation. Only 100 μ l of the 1000 μ l of the transformed cells was plated on LB with carbenicillin plates (carbenicillin at 50 μ g/ml). The plates were incubated at 37°C overnight. Twelve individual colonies were picked for miniprep analysis. Several diagnostic digests confirmed the presence of the heavy chain insert. Plasmid DNA was isolated on a CsCl gradient (Sambrook et al., supra). The nucleotide and amino acid residue

sequences are illustrated in Figures 27A through 27E and the nucleotide and amino acid residue sequences are given in the sequence listing as SEQ ID NOs 154 and 155.

5

e. Insertion of the b12 Light Chain into the pEE12 Mammalian Expression Vector

The light chain was ligated into the pEE12 vector (Celltech, England) from the pSG-5 vector involving similar steps as described in Example 4d for the heavy chain. The pEE12 vector has a human CMV promoter for expression of the light chain, a polylinker to provide cloning sites, and a polyadenylation signal for termination of transcription. The vector also contains the GS selectable marker gene, whose expression is controlled by an SV40 early promoter at the 5' end of the GS gene, an intron, and a polyadenylation signal at the 3' end of the GS gene.

20

1) Preparation of Modified b12 Light Chain

The 5' PCR primer was designed to replace the EcoR I cloning site with a HindIII cloning site. The 3' PCR primer maintained the EcoR I cloning site.

25

The primer pair, LC-5 (SEQ ID NO 167) and LC-2 (SEQ ID NO 165), was used in the PCR reaction as described in Example 4a1 to amplify the Fab b12 light chain gene and incorporate HindIII and EcoR I cloning sites into 5' and 3' ends of the fragment, respectively. The b12 pSG-5 vector containing the b12 light chain was used as the template in the PCR reaction. For the PCR reaction, 1 μ l containing 100 ng of b12 pSG-5 DNA was admixed with 10 μ l of 10X PCR buffer in a 0.5 ml microfuge tube. To the DNA admixture, 8 μ l of a 2.5 mM solution of dNTPs (dATP, dCTP, dGTP, dTTP) was admixed to result in a

30

35

final concentration of 200 micromolar (μM) of each dNTP. 1 μl (equivalent to 20pM) of the LC-5 primer and 1 μl (20 pM) of the 3' backward LC-2 primer was admixed into the DNA solution. To the admixture, 73 μl of sterile water and 2.5 units of Taq DNA polymerase was added. Two drops of mineral oil were placed on top of the admixture and 35 rounds of PCR amplification in a thermocycler were performed. The amplification cycle consisted of 52°C for 1 minute, 72°C for 2 minutes and 94°C for 0.5 minutes.

The resultant PCR modified b12 light chain DNA fragment was purified by electrophoresis in a 2.5% Nu-Sieve agarose gel (FMC). The area in the agarose containing the modified b12 light chain DNA fragment was isolated from the agarose.

2) Insertion of the Modified b12 Light Chain into the pEE12 Mammalian Expression Vector

The modified b12 light chain purified PCR product and the pEE12 vector were digested with HindIII and EcoR I in separate reactions. The digested DNAs were analyzed on an LMPA gel, the DNA excised, and ligated together in the presence of the LMPA gel as described for the heavy chain construct in Example 4d. The ligation products were transformed into DH5 α competent cells, minipreps analyzed, and DNA prepared as described for the heavy chain constructs in Example 4d.

f. Insertion of the Modified b12 Heavy Chain into the pEE12 Mammalian Expression Vector Containing the b12 Light Chain to Create the Combinatorial Vector pEE12 Combo BM 12

A heavy chain cassette comprising the

HCMV promoter, enhancer elements, heavy chain gene, and polyadenylation signal were removed from the pEE6 vector and inserted into the pEE12 vector containing the b12 light chain gene, prepared in Example 4e, to generate the combinatorial construct, pEel2 Combo BM 12, containing both the b12 light and heavy chain genes (Figure 28).

The heavy chain cassette was removed from the pEE6 vector by digestion with BglII and Sal I. The pEE12 vector containing the light chain gene, prepared in Example 4e, was also digested with BglII and Sal I. The heavy chain cassette and the pEE12 vector containing the light chain gene from Example 4e were ligated together at the BglII and Sal I sites as described in Example 4d. The combinatorial construct was transformed into DH5 α competent cells and miniprep DNA was analyzed for the presence of the heavy and light chains as in Example 4d. The nucleotide sequence of the heavy and light chain genes was determined. The nucleotide sequence of pEel2 Combo BM 12, the pEE12 vector containing the b12 heavy and light chain genes is given in the sequence listing as SEQ ID NO 156 and is illustrated in Figures 29A through 29R.

g. gp120 Binding of b12 IgG1 Antibody
Expressed from the Heavy and Light Chain
Genes in the Combinatorial Vector pEel2
Combo BM 12

The combinatorial pEel2 Combo BM 12 vector containing both the heavy and light chain genes was used to transfect CHO cells. Stable clones were selected in Glasgow Minimal Essential Media (GIBCO) supplemented with 10% dialyzed fetal bovine serum and 50 μ M methyl sulfoxamine (MSX). Several clones were isolated and expanded in 6-well cluster dishes. The supernatants of subconfluent cultures were harvested and tested by ELISA for

binding to gp120 as described in Example 4c2. The clone producing the highest levels of b12 IgG1 as determined by ELISA with gp120 IIIB was chosen for further study. The antibody was purified by affinity chromatography using protein A as described in Sambrook, et al., supra. The affinity of b12 IgG1 for gp120 IIIB as measured by surface plasmon resonance as described in Example 2b6c is $1.3 \times 10^9 \text{ M}^{-1}$.

5. Neutralizing Activity of Recombinant b12 Whole IgG1 Antibody (b12 IgG1) Against HIV-1 In Vitro

The key issue in producing antibodies to HIV-1 for therapeutic or prophylactic purposes is that they should be highly potent (of high affinity and neutralizing ability) and be cross reactive with a wide range of primary clinical (field) isolates. These are generally two opposing characteristics. The ability of b12 whole IgG1 antibody (b12 IgG1) to neutralize the infectivity of laboratory strains of HIV-1 and a wide variety of primary clinical isolates has been examined in p24 ELISA assays, microplaque assays, and by syncytial formation assays.

The primary clinical isolates used as a source of HIV-1 virus in these assays came from various regions of the world by three organizations: the World Health Organization (WHO), the Henry M. Jackson Foundation for the Advancement of Military Medicine (HMJFAMM), and the National Institute of Allergy and Infectious Diseases (NIAID). Isolates from the WHO Network for HIV-1 Isolation and Characterization were obtained through the AIDS Research and Reference reagent Program, Division of AIDS, NIAID, NIH. Isolates from HMJFAMM were provided by Dr. John Mascola, Walter Reed Army Institute of Research, Rockville, MD and Dr.

Francine McCutchan, Henry M. Jackson Research Laboratory, Rockville MD. Isolates from NIAID were kindly provided by Dr. Jim Bradac, Division of AIDS, NIAID, NIH.

5 The HIV-1 viruses were collected from various regions of the world, expanded in mitogen-stimulated peripheral blood mononuclear cells (PBMC) (Mascola et al., J. Infect. Dis., 169:48-54 (1994)), and culture supernatants containing
10 infectious virus were stored in central repositories at -70°C. The designation of viruses into clades was made on the basis of sequence information based on the gag gene or on the V2-C5 region of gp120, or in some cases, after
15 heteroduplex mobility analysis (Louwagie et al., AIDS, 7:769-772 (1993) and Delwart et al., Science, 262:1257-1261 (1993)).

 The HIV-1 viruses include a set of 14 primary isolates which contain a high proportion of
20 isolates which are relatively refractory to antibody neutralization by sera from other HIV-1 infected individuals (Wrin et al., J. Acq. Imm. Def. Synd., 7:211-219 (1994)), 12 primary infant isolates obtained at birth or within two weeks of
25 age, and 69 international isolates belonging to 6 different clades.

 Several different neutralization assays were performed because HIV-1 neutralization by antibody shows considerable variation depending upon the
30 assay used and the precise experimental conditions such as inoculum size and incubation time of virus and antibody (D'Souza et al., AIDS, 8:169-173 (1994)). By performing neutralization assays on a range of laboratory and primary isolates in a
35 number of different laboratories, it has been demonstrated that b12 IgG1 is a highly potent neutralizing antibody effective against a wide breadth of isolates.

a. Quantitative Neutralization of HIV-1 MN
and IIIB by b12 IgG1 as Measured in a
Plaque Assay

b12 IgG1 was initially tested for its
ability to neutralize the HIV-1 laboratory strains
MN and IIIB in a plaque formation assay in
laboratories which recently tested a panel of
monoclonal antibodies as part of the NIAID/WHO
Antibody Serological Project (D'Souza et al.,
supra).

b12 IgG1 showed 50% neutralization titers of 3
ng/ml for the MN strain and 7 ng/ml for the IIIB
strain using plaque formation (Hanson, et al., J.
Clin. Microbiol., 28:2030-2034 (1990)) to determine
the ability of the antibody to inhibit infectivity
of the HIV-1 strains.

b. Quantitative Neutralization of HIV-1 MN
and IIIB by b12 IgG1 as Measured by
Syncytial Formation

b12 IgG1 showed 50% neutralization titers
of 20 ng/ml for both MN and IIIB strains using
syncytial formation as the reporter assay as
described in Example 3b (Nara et al., AIDS Res.
Human Retroviruses, 3:283-302 (1987)).

The syncytial formation assay was performed as
described in Example 5c. Briefly, virus was grown
in H9 cells. For infectivity measurement,
monolayers of CEM-SS target cells were cultured
with 100-200 syncytial forming units (SFUs) of
virus, in the presence or absence of antibody, and
the number of syncytia determined after 3-5 days of
incubation. The assays were repeatable over a
virus-surviving fraction range of 1 to 0.001 within
a 2 to 4-fold difference in the concentration of
antibody ($P < 0.001$).

c. Neutralization of Primary Virus Isolates
by b12 IgG1 as Measured by the p24 ELISA
Assay

5 The ability of b12 IgG1 to neutralize
infectivity of PBMCs by HIV-1 virus was
quantitatively measured in the p24 ELISA assay
(Daar et al., Proc. Natl. Acad. Sci. U.S.A.,
87:6574-6578 (1990) and Ho et al., J. Virol.,
65:489-493 (1991)). The p24 ELISA assay is further
10 described in Example 3a.

1) Neutralization of Ten Primary Virus
Isolates by b12 IgG1

15 HIV-1 viruses were isolated from 10
individuals from various locations in the U.S. and
with varying disease status. The HIV-1 viruses had
been cultured only once or twice in peripheral
blood mononuclear cells (PBMCs). Viral stocks were
grown in PBMCs and the assay was performed in
20 PBMCs.

Briefly, HIV-1 virus at 50 TCID₅₀ and varying
concentrations of b12 IgG1 were incubated together
for 30 min at 37°C before addition to PHA-
stimulated PBMCs. HIV-1 virus replication was
25 assessed after incubation for 5 to 7 days by p24
ELISA measurement as described in Example 3a. HIV-
1 virus positive controls used in this assay were
the molecularly cloned HIV-1 virus JR-CSF and the
HIV-1 isolate JR-FL (O'Brien et al., J. Virol.,
30 66:3125-3130 (1992), O'Brien et al., Nature,
348:69-73 (1990), and O'Brien et al., J. Virol., in
press (1994)). Stocks of JR-CSF were prepared by
infection of PBMC with supernatants initially
obtained by DNA transfection. HIV-1 IIIB and HIV-1
35 MN are viruses with an extensive history of passage
in transformed T-cell lines (Robert-Guroff et al.,
Nature, 316:72-74 (1985)). Stocks of these strains
grown in H9 cells were passaged in mitogen-

stimulated PBMC to prepare viruses that had been grown in the same cells as the primary viruses, to eliminate the influence of any host cell-dependent epigenic factors on virus neutralization (Wrin, et al., J. Acq. Imm. Def. Synd., 7:211-219 (1994)). The stock of PBMC-grown MN was a gift from A. N. Conley (Merck Research Labs).

2) Neutralization of 12 Primary Infant Isolates by b12 IgG1

b12 IgG1 was also tested for the ability to neutralize infectivity of a panel of 12 primary infant isolates in the p24 ELISA assay. Virus isolates were obtained from 12 infants born to HIV-1 seropositive mothers; 7 were obtained at birth and 5 between birth and 14 days of age. All the infants were from California. Virus was isolated from patient PBMCs by coculture with PBMCs from healthy seronegative donors. Viral stocks were prepared by passaging the last positive culture dilution once into PBMCs. All of the isolates, except one (isolate 7), were non-syncytial inducing in MT2 cells and therefore could not be assayed in the syncytial forming assay as herein described. HIV-1 virus from these stocks was grown in PBMCs and neutralization assessed using PHA-stimulated PBMCs as indicator cells and determination of extracellular p24 as the reporter assay essentially as described in Example 3a (AIDS Clinical Trials Group Virology manual for HIV Laboratories, Department of AIDS Research, NIAID, NIH, version 2.0 (1993)).

Serial dilutions of b12 IgG1 (0.3 to 20 μ g/ml) were incubated with 20 TCID₅₀ or 100 TCID₅₀ virus in triplicate for 2 hours at 37°C before addition to PHA-stimulated PBMCs. Virus replication was assessed after 5 days by p24 ELISA measurement. Neutralization was expressed as either a 50% or 90%

reduction in p24 antigen as compared to values observed in the absence of antibody (Table 6).

5 d. Neutralization of Primary Virus Isolates
 by b12 IgG1 as Measured in a Microplaque
 Assay

 A quantitative microplaque assay to
measure the reduction of infectivity of primary
clinical isolates of HIV-1 in the presence of the
10 b12 IgG1 and pooled human plasma was performed as
described in Hanson et al., J. of Clin. Microb.,
2030-2034 (1990). The set of primary clinical
isolates was chosen to contain a high proportion of
isolates which are relatively refractory to
15 antibody neutralization by sera from other HIV-1
infected individuals (Wrin et al., J. Acq. Imm.
Def. Synd., 7:211-219 (1994)). Viruses were grown
in PBMCs and the assay carried out in MT2 cells.
This limits study to viruses which grow in this
20 cell line but provides an additional measure of
neutralization.

 Primary clinical isolates of HIV-1 were
isolated from frozen peripheral blood lymphocytes
obtained from seropositive donors as described in
25 Gallo et al., J. of Clin. Microb., 1291-1294 (1987)
and cultivated in peripheral blood mononuclear
cells (PBMC). Briefly, HIV isolates were obtained
by incubating frozen HIV-infected patient PBMCs
with seronegative donor PBMCs in RPMI-1640 medium
30 containing 20% heat-inactivated fetal bovine serum,
2 µg/ml polybrene, 5% interleukin-2, and 0.1% anti-
human leukocyte interferon. The cultures were fed
with fresh donor PBMCs once a week, and the
supernatants were assayed for the presence of
35 reverse transcriptase (RT) activity beginning at
day 11. The cultures were considered positive if,
for 2 consecutive weeks, the RT counts were >10-
fold higher than those in the cultures of the

seronegative donor PBMCs alone.

The resultant RT-positive virus isolates were tested for cytolysis in the MT4 (α -4 clone) (Hanson et al., supra). Cytolysis in MT4 is a requirement for viruses to be usable in the subsequent MT2 microplaque assay system. Supernatant fluids from the primary PBMC isolation cultures were used to infect expanded cultures of phytohemagglutinin (PHA)-stimulated PBMCs from healthy seronegative blood donors. These infected PBMC cultures were grown in RPMI-1640 medium supplemented with 15% fetal bovine serum, 5% interleukin-2, 0.1% anti- α interferon, 2 μ g/ml polybrene, 50 μ g/ml gentamicin, 100 U/ml penicillin, and 100 μ g/ml streptomycin. The crude supernatants were harvested after 7 days and frozen as viral stocks at -70°C.

The primary clinical isolates of HIV-1 used in this microplaque assay are given in Table 6. VL134, VL648, and VL025 are viruses isolated from infected mothers in New York in 1992; UG266 and UG274 are clade D isolates which were a gift from John Mascola the Division of Retrovirology, Walter Reed Army Institute of Research; the remaining viruses were isolated from homosexual males in California in 1992. The pooled human plasma preparation, containing neutralizing antibody, was derived from 13 HIV-1 positive individuals selected for high neutralization titer against the MN isolate. The laboratory HIV-1 strains MN and IIIb were propagated in H9 cells as controls in the microplaque assay.

b12 IgG1 and a pool of human plasma from 13 HIV-1 seropositive patients were used as the source of neutralizing antibodies in a 96-well microtiter plaque reduction assay as described by Hanson et al., supra. Briefly, 3-fold serial dilutions of the b12 IgG1 or heat-inactivated pooled patients' plasma were combined in quadruplicate with an equal

5 volume containing 20 plaque-forming units (PFU) of
HIV-1 virus per well and incubated for 18 hours at
37°C. Negative control wells also contained 50%
normal human serum pool with no patient immune
serum. After the 18 hour incubation of Fabs or
serum and virus, 90,000 MT2 cells were added per
well and incubated at 37°C for 1 hour. SeaPlaque
Agarose in assay medium at 39.5°C was then added to
10 a final concentration of 0.8%. While the warm
agarose was still molten, the microtiter plates
were centrifuged at 20°C for 20 minutes at 500 X g
to form cell monolayers. The plates were incubated
for 6 days at 37°C and then stained 18 to 24 hours
with 50 µg/ml propidium iodide. The fluorescent
15 plaques were counted with transillumination by a
304 nm ultraviolet light source using a low-power
stereo zoom microscope. Inhibition of infectivity,
or neutralization titer, is defined as the µg/ml of
Fab or the plasma dilution giving 50% inhibition of
20 plaque count as compared with controls without
antibody. This dilution was interpolated between
data points.

25 e. Results of the Neutralization Assays by
b12 IgG1 with Laboratory Virus Isolates
Results of the ability of the b12 IgG1 to
neutralize laboratory virus isolates in both the
plaque and syncytial formation assays suggest the
antibody is approximately two orders of magnitude
30 more potent than other CD4 site antibodies in the
WHO/NIAID Project and comparable to the best
antibodies directed to the V3 loop of gp120.
However, whereas antibodies directed to the V3 loop
of gp120 are strongly strain specific, b12 IgG1 is
35 roughly equally effective against MN and IIB. The
b12 IgG1 antibody is comparable in potency to a
CD4-IgG molecule in these assays (Example 3c). In
a separate assay using p24 production to determine

infectivity (Daar et al., Proc. Natl. Acad. Sci. U.S.A., 87:6574-6580 (1990) and Ho et al., J. Virol., 65:489-493 (1991)), 50% neutralization titers of less than 40 ng/ml were found for both the MN and IIIB laboratory strains.

f. Results of the Neutralization Assays by b12 IgG1 with Primary Virus Isolates

b12 IgG1 showed essentially complete neutralization of 7 of 10 isolates at 5 μ g/ml with all the isolates showing 50% neutralization at \leq 1 μ g/ml as determined in the p24 reporter assay (Figure 21).

The inhibition of infectivity, or neutralization titer, for b12 IgG1 and the pooled HIV seropositive human plasma from 13 donors is given in Table 6. The neutralization titer for each of the viral isolates is expressed as the minimum μ g/ml of b12 IgG1 required for 50% inhibition of plaque count as compared to the controls. The neutralization titer for each of the viral isolates is expressed as the minimum titer of the pooled HIV seropositive human plasma from 13 donors required for 50% inhibition of plaque count as compared to the controls.

Table 6

		host	b12 IgG1 50% neutralization titer ($\mu\text{g/ml}$)	pooled human plasma: dilu- tion for 50% neutralization
5	<u>virus</u>	<u>cell</u>		
	IIIB	H9	0.007	1:767
	MN	H9	0.003	1:24,000
10	VL135	PBMC	10	1:44
	UG274	PBMC	0.7	1:37
	VL134	PBMC	5.6	1:30
	VL596	PBMC	8.5	1:17
	UG266	PBMC	3.8	1:12
15	VL434	PBMC	22	1:10
	VL172	PBMC	>200	1:10
	VL750	PBMC	>200	1:10
	VL069	PBMC	>50	<1:10
	VL077	PBMC	>200	<1:10
20	VL114	PBMC	<7.4	<1:10
	VL263	PBMC	5.0	<1:10
	VL648	PBMC	16.7	<1:10
	VL025	PBMC	16.7	<1:10

25 The b12 IgG1 was able to neutralize ten of the
fourteen primary clinical isolates assayed at
concentrations of $\leq 50 \mu\text{g/ml}$ as measured as the $\mu\text{g/ml}$
required for 50% inhibition of plaque count as
compared to the controls (Table 6). Pooled human
30 plasma was able to neutralize 5 of the 14 primary

clinical isolates assayed at >1:10 dilution as measured as the dilution required for 50% inhibition of plaque count as compared to the controls without antibody.

5 Table 6 shows that four isolates, which were not neutralized even by a 1:10 dilution of pooled human plasma, were neutralized by b12 IgG1. Most of the viruses reported in Table 6 were isolated from U. S. donors although two, both of which are neutralized by
10 b12 IgG1, were from Ugandan donors and assigned to clade D.

Results of neutralization of 12 infant primary isolates with b12 IgG1 as determined by p24 ELISA measurements are given in Table 7.

15

Table 7

		b12 IgG1	
		Antibody Concentration ($\mu\text{g/ml}$)	
	<u>Infant Isolate</u>	<u>50% inhibition</u>	<u>>90% inhibition</u>
20	1	20	>20
	2	1.25	>20
	3	<0.3	0.3
	4	<0.3	0.6
	5	2.5	20
	6	5	>20
25	7	5	>20
	8	<0.3	0.3
	9	0.3	5
	10	0.3	2.5
30	11	<0.3	0.6
	12	<0.3	0.3

As shown in Table 7, b12 IgG1 achieved 90% neutralization for 8 of 12 infant isolates at concentrations of ≤ 20 $\mu\text{g/ml}$ in the p24-based assay. All 12 isolates were 50% neutralized in the range of 0.3 to 20 $\mu\text{g/ml}$ with the majority being neutralized at < 5 $\mu\text{g/ml}$. In contrast, a pooled hyperimmune globulin product HIVIG achieved 90% neutralization of only 3 or 12 isolates within a concentration range up to 100 $\mu\text{g/ml}$. HIVIG is a hyperimmune IgG preparation obtained from the pooled plasma of selected HIV-1 asymptomatic seropositive donors meeting the following criteria: presence of p24 serum antibody titers > 128 , CD4 lymphocyte count ≥ 400 cells/ μl and the absence of p24 and hepatitis B surface antigen by enzyme immunoassay (Cummins et al., Blood, 77:1111-1114 (1991)). The HIVIG used in these experiments was lot number IHV-50-101 (North American Biologicals).

HIV-1 neutralization by antibody shows considerable variation depending upon the assay used and precise experimental conditions such as inoculum size and incubation time of virus and antibody (D'Souza et al., supra). However, by carrying out neutralization on a range of laboratory and primary isolates in a number of assays in different laboratories, we have shown that b12 IgG1 is a highly potent neutralizing antibody effective against a wide breadth of primary isolates. The results clearly demonstrate that, although primary isolates may be more difficult to neutralize by antibody than laboratory strains, they are not intrinsically resistant (Conley et al., Proc. Natl. Acad. Sci., U.S.A., 91:3348-3353 (1994)). The potency of b12 IgG1 against the majority of U. S. isolates is in a concentration range (≤ 5 $\mu\text{g/ml}$) which could be achieved in vivo in passive immunotherapy. Furthermore, the affinities of recombinant

antibodies displayed on phage can be enhanced by mutagenesis and selection in vitro and this strategy has been used to considerably improve the potency and breadth of reactivity of Fab b12 (Barbas et al., Proc. Natl. Acad. Sci., U.S.A., 91:3809-3812 (1994)). For optimal potency and strain cross-reactivity for passive immunization, a cocktail of in vitro improved antibodies may be most appropriate.

The results have implications for passive immunization and vaccine design. The ability of b12 IgG1 to neutralize a range of primary isolates implies conservation of a structural feature associated with the CD4 binding site of gp120 which is accessible to antibody and important for neutralization. A vaccine might seek to present this feature to the immune system. Clearly, the feature is present on recombinant gp120 since b12 was affinity selected from a library using this molecule. However, b12 and related antibodies formed only a small part of the repertoire affinity selected from this library by recombinant gp120. Most of the antibodies obtained were far less potent in neutralization even though they were also directed to the CD4 binding site, were cross-competitive with b12 for binding to recombinant gp120 and had similar affinities to b12 (Barbas et al., Proc. Natl. Acad. Sci., U.S.A., 89:9339-9343 (1992), Barbas et al., J. Mol. Biol., 230:812-823 (1993), and Example 2b6)(c)). Therefore, recombinant gp120 appears to present the b12 epitope in conjunction with several other weakly neutralizing and overlapping epitopes and its efficacy as a vaccine may suffer. Interestingly, evidence from antibody binding to infected cells suggests that b12 does recognize a native conformation of gp120 more effectively than other CD4 binding site antibodies (Example 7). In any

case, b12 IgG1 and the library approach could be useful in vaccine and passive immunization evaluation. The ability of a candidate vaccine to preferentially bind b12 and/or preferentially select potent neutralizing antibodies from libraries should be positive indicators for vaccine development.

5. Determination of the Relationship Between the Epitopes Recognized by Fabs with Purified HIV-1 Antigens

The Fabs show a spectrum of neutralizing abilities as described in Example 5. It was therefore sought to determine if the epitopes recognized by individual Fabs could be distinguished from each other, and if possible, determine how the epitopes recognized by the individual Fabs related to neutralization.

a. Competitive ELISA between Fabs and b13 Whole IgG1 Antibody for Binding to gp120

The first method to distinguish between the epitopes bound by the Fabs of this invention was to compare the epitope recognized by the Fab b13 with the other Fabs. The Fab b13 had been spliced to the Fc region of IgG1 to generate a whole IgG1 molecule and therefore contains the Fc region of the IgG1 antibody. The other Fabs do not contain the Fc region of the IgG1 antibody. The binding of the b13 IgG1 could therefore be distinguished from the binding of other Fabs by using a labeled anti-Fc reagent in competition ELISA. A competition ELISA in which the Fabs b3, b6, b11, b12, and b14 competed with b13 IgG1 for binding to immobilized gp120 was performed.

Competitive ELISAs were performed between the Fabs b3, b6, b11, b12, and b14 and the b13 whole IgG1 antibody. The whole antibody was obtained by

splicing constant domain genes with the b13 Fab and expressing the protein in Chinese Hamster Ovary cells (CHO) as described in Example 4g (Bender et al., supra and in Example 4a for the Fab b12). The ELISA was performed as described above in Example 2b6) (b). Briefly, microtiter wells were coated with 0.1 µg/ml of gp120 derived from the HIV-1 strain LAI in 0.1 M bicarbonate buffer at pH 8.6. Soluble or free Fab fragments were serially diluted from 1:100 to 1:32,000 in 0.5% BSA/0.025% Tween 20/PBS. The dilution of b13 IgG1 was held constant at 1:10,000 in 0.5% BSA/0.025% Tween 20/PBS. The b13 IgG1 and Fabs were admixed, added to the gp120-coated microtiter wells and maintained for 120 minutes at 37°C. After maintenance, the wells were carefully washed ten times with 0.05% Tween 20/PBS. The amount of b13 IgG1 antibody bound to the plate after washing was detected using a peroxidase-labeled antibody specific for the Fc portion of IgG1 contained on the b13 antibody.

Results of this assay indicated that the Fabs b3, b6, b11, b12, and b14 are competitive with b13 IgG1 for binding to gp120 indicating that the epitopes recognized by the individual Fabs are probably either proximal or identical to the epitope recognized by the b13 IgG1. A control anti-tetanus toxoid Fab did not compete with IgG1 b13 in this assay.

Competition monitored in an ELISA format showed that all of the Fabs compete with the b13 Fab as a whole IgG. There is also an indication that Fabs b12 and b13 are distinct in that they are somewhat less effective in cross-competition than the other members of the panel.

b. Epitope Similarity Determination
Between the Fabs in Binding to gp120
Using BIAcore

A more precise method for determining the similarity of epitopes was performed using the BIAcore. The procedure adopted here was to immobilize a polyclonal anti-human F(ab')₂ on the sensor chip and use this to capture the individual Fabs. An Fab of this invention was injected and captured by the polyclonal anti-human F(ab')₂. The captured Fab was then used to bind gp120 derived from the HIV-1 strain LAI. The captured Fab would thus bind the gp120 at its respective epitope. A second Fab of this invention was then injected. A response in the BIAcore assay after injection of the second Fab indicates that binding has occurred. If the second Fab injected recognizes the same or similar epitope on the gp120 as the first Fab, no response would occur. No response would therefore indicate that the two Fabs tested in the assay competed for binding to the same or similar epitope on gp120. Alternatively, a response in the assay suggests that the epitopes recognized by the two Fabs are distinct from one another and that binding of the second Fab to gp120 to a second epitope is possible in the presence of the first Fab. A response would therefore indicate that the two Fabs tested in the assay did not compete for binding to the same or similar epitope.

The precise epitope similarity determination with the BIAcore was performed as follows. A flow rate of 5 μ l/min of PBS, pH 7.4 was established and the biosensor chip was activated by injecting 30 μ l of activation solution (Pharmacia Biosensor, 50% 0.2 M N-ethyl-N'-(e-diethylaminopropyl)-carbodiimide, 50% N-hydroxysuccinimide). The flow rate was then adjusted to 10 μ l/min and the antigen was injected in 10 mM sodium acetate buffer, pH 4.5. Forty μ l of goat anti-human F(ab')₂ (Pierce) at a concentration of 40 μ g/ml in 10 mM sodium acetate buffer, pH 4.5 was injected to give a final

immobilization of 10000 Response Units (RU). The chip was then blocked from any further immobilization by injecting 30 μ l of 1 M ethanolamine, pH 8.5 (Pharmacia Biosensor). The flow rate was adjusted to 1 μ l/min and 4 μ l of the first Fab at a concentration of 100 μ g/ml was injected, immediately followed by 4 μ l of an anti-cytomegalovirus Fab at a concentration of 150 μ g/ml to block any remaining binding sites on the immobilized goat anti-human F(ab')₂. Next, 4 μ l of gp120 at a concentration of 10 μ g/ml was injected followed by 4 μ l of the second Fab at 100 μ g/ml. The assay was performed with a combination of all of the Fabs to give a mosaic of binding patterns. The entire surface was regenerated with 25 μ l of 60 mM HCl so that the next cycle could be performed.

Table 8b indicates the results of the epitope similarity determination by BIAcore. Table 8a shows the positive and negative controls for the clones used. The positive controls are the RU levels obtained when the first Fab used is the clone indicated and the second Fab is an anti-gp120 V3-loop Fab. The Fabs of this invention compete with soluble CD4 for binding to gp120. The second Fab, an anti-gp120 V3-loop Fab, neither competes with soluble CD4 nor competes with anti-CD4 site Fabs and therefore would react with a different epitope than the Fabs of this invention. As can be seen from the table, all positive controls result in significant values of 125 or more, indicating the validity of the technique to distinguish between non-identical epitopes. The negative controls are the values obtained when the same Fab is injected twice. This gives the background values for each Fab. These values were subtracted from all subsequent experiments in order to give true values.

An epitope map, Table 8b, was then constructed. ND indicates that this combination of Fabs was not performed. It can be seen from this map that Fabs b3, b6, b11, and b14 form a set which compete highly effectively with one another for binding to a similar or the same epitope. For the most part, a member of the set competes for binding as well with another member as it does with itself (RU = 0). On the other hand, b12 and b13 appear somewhat different in that while they compete for binding with members of the above set, they do not compete as effectively as the other Fabs within the set. Further, competition for binding to the same or similar epitope between b12 and b13 is incomplete. This suggests that the epitopes of Fabs b12 and b13 are sufficiently dissimilar from those of the other four and from each other, to allow detectable binding when they are used in combination with any of the other Fabs. It may therefore be concluded that clones b3, b6, b11, and b14 bind the same or similar epitopes, with Fabs b12 and b13 bind to epitopes which can be distinguished from the other epitopes in this assay.

Table 8a

Fab	b3	b6	b11	b12	b13	b14
POSITIVE						
CONTROL (RU)	129	128	131	125	135	134
NEGATIVE						
CONTROL (RU)	24	38	ND	17	15	ND

ND indicates that this combination of Fabs was not performed.

Table 8b

Fab 1

		<u>b13</u>	<u>b12</u>	<u>b6</u>	<u>b3</u>
5					
	b14	30	24	14	0
	Fab 2				
	b11	54	28	14	0
	b3	26	29	0	0
	b6	21	17	0	ND
	b12	22	0	ND	ND

ND indicates that this combination of Fabs was not performed.

c. Comparison of Fab Epitopes with Wild-type and Mutant Forms of gp120 Using ELISA with gp120 in the Solid Phase

Epitope similarity determinations of the panel of Fabs was performed with a panel of HXBc2 gp120 mutants of the HIV-1 strain LAI. Conserved residues of gp120 were altered to generate the HXBc2 gp120 mutants. The interaction between the mutants and Fabs was investigated to examine binding specificity differences between the Fabs at greater resolution. The HXBc2 gp120 mutants used in this assay had been previously characterized with respect to gp160 precursor processing, gp120-gp41 association, and CD4 binding ability (Olshevsky et al., J. Virol., 64: 5701-5707 (1990)). Both wild type and mutant gp120s were tested for their ability to bind a saturating concentration of each Fab.

The epitope determination with wild-type and mutant gp120 was performed with HIV-1 envelope glycoproteins from culture supernatants of COS-1 cells transfected with plasmids expressing either wild-type or mutant gp120 from the HXBc2 clone.

Microtiter wells were coated with the antibody D7324 (Aalto BioReagents; Dublin, Ireland) which binds to the conserved 15 amino acid sequence at the carboxy terminus of gp120. The wild-type or mutant gp120 were thus captured onto the surface of microtiter wells by binding to the D7324 antibody. A reference HIV-1 positive human serum pool at a 1:3000 dilution in 0.5% Tween 20 was assayed for binding to the wild-type and mutant gp120s by incubating the serum pool with the immobilized gp120. The bound antibody was detected by a second enzyme conjugated antibody. The reading obtained with the HIV-1 positive human serum pool, N=4, was used as the reference value for each mutant. The Fabs of this invention were then assessed for binding to the wild-type and mutant gp120s and the ratio of the Fab to reference serum was determined for each gp120 mutant (Table 9). The average ratio for the entire panel of Fabs was calculated and any individual ratio deviating from the mean by less than 0.5 times was considered to indicate a gp120 amino acid change that decreased Fab recognition, while those deviating by more than 2.0 times indicated an amino acid change that enhanced Fab recognition. In this way, a map of mutations affecting the binding of the Fab to gp120 was obtained for each clone essentially as previously described (Helseth et al., *J. Virol.*, 65:2119-2123 (1991) and Olshevsky et al., *supra*).

Table 9

	Mutation	Fab					
		<u>B3</u>	<u>B6</u>	<u>B11</u>	<u>B12</u>	<u>B13</u>	<u>B14</u>
	45 W/S	1.60	0.61	0.50	0.68	1.20	0.28
35	113 D/A	1.46	1.73	1.89	1.13	0.99	0.00

	Mutation	Fab					
	113 D/R	1.40	1.50	1.61	0.67	0.71	0.00
	NO V1/V2	1.07	1.48	1.42	0.23	0.86	1.68
	NO V1/V2/V3	2.05	1.48	1.94	0.47	0.95	1.60
	NO V3	1.88	1.64	1.92	0.46	1.08	1.72
5	183/184 PI/SG	0.82	0.73	0.69	0.33	0.92	0.32
	207 K/W	1.15	1.57	1.19	2.54	1.30	1.36
	252 R/W	1.58	1.52	1.58	1.65	1.39	2.04
	256 S/Y	0.64	0.14	0.33	0.82	1.15	0.00
	257 T/R	0.08	0.59	0.00	0.76	0.22	0.00
10	257 T/A	0.86	0.93	0.75	0.99	0.68	0.40
	257 T/G	0.91	0.70	1.14	0.74	0.75	0.00
	262 N/T	1.06	0.64	1.19	0.62	0.72	0.24
	269 E/L	0.73	0.48	0.45	0.78	0.83	0.20
	314 G/W	0.59	0.36	0.39	0.65	0.71	0.28
15	356 N/I	0.67	0.66	0.39	0.92	0.80	0.52
	368 D/R	0.19	0.18	0.00	0.04	0.00	0.00
	368 D/T	0.28	0.20	0.00	0.03	0.02	0.00
	370 E/R	0.01	0.25	0.17	0.07	0.00	0.00
	370 E/Q	0.25	0.89	0.58	0.46	0.14	0.00
20	384 Y/E	1.21	1.02	1.11	0.25	0.02	0.88
	386 N/Q	0.88	0.59	0.31	1.05	0.01	0.36
	395 W/S	0.92	0.59	0.47	1.00	1.05	0.12
	427 W/S	1.57	1.11	1.53	0.63	0.98	0.00
	435 Y/S	1.93	1.16	1.58	1.41	1.24	2.04
25	450 T/N	0.62	0.48	0.58	0.75	0.75	0.60

	Mutation	Fab					
	457 D/A	0.62	0.39	0.44	0.28	0.62	0.20
	457 D/R	0.84	0.55	0.92	0.32	0.58	0.56
	470 P/L	0.80	0.64	0.72	0.72	0.18	0.24
	475 M/S	0.06	1.02	0.33	1.50	1.39	0.92
5	477 D/V	0.50	0.09	0.00	0.07	0.52	0.00

10 The general patterns observed are broadly similar to many CD4 site antibodies and of soluble CD4. Fab b12 is distinguished by its decreased binding to a mutant in which the V1 and V2 loops are deleted. This may or may not be related to the enhanced neutralizing ability of Fab b12. However, it is clear that the V1 and V2 loops and the V3 loop can affect antibody binding to the CD4 binding site either by direct contact or by transmitted conformational effects.

15 Sensitivity to certain mutations in residues, particularly towards the C-terminus of gp120, has previously been associated with CD4 binding site antibodies (Thali et al., J. Virol., 66:5636-5641 (1992) and Thali et al., J. Virol., 65:6188-6193 ((1991)). These mutations include residue 257 mutated from threonine to arginine (257 T/R), 368 D/R, 370 E/R, 457 D/A and 477 D/V. Most of these mutations abrogate Fab binding or reduce it to low levels consistent with the assignment of the recombinant Fabs in this assay as reacting with the CD4 site.

20 In a particular mutant of gp120, the V1/V2 loop (residues 119-205) is completely removed. This mutation enhances the binding of Fabs b6, b11, and b14 but significantly decreases the binding of Fab b12. Deletion of the V3 loop produces a more modest decrease in Fab b12 binding while generally

enhancing the binding of the other Fabs. The 314 G/W change in the V3 loop produces a decrease in binding of all the Fabs. This effect has been observed for other CD4 binding site antibodies (Moore and Sodroski, unpublished observations).

When the binding specificities of each Fab is examined in detail, each Fab has a unique mutant binding profile. For example, Fab b14 binding is eliminated by the 113 D/A change whereas the binding of the other Fabs is unchanged or enhanced; Fab b3 and b11 binding is reduced by the 475 M/S mutation but binding by the other Fabs is unchanged and the 370 E/Q change reduces binding of all the Fabs except for b6 and possibly b11. Fab b12 is distinguished by its decreased binding to a mutant in which the V1 and V2 loops are deleted. This may or may not be related to the enhanced neutralizing ability of Fab b12 and will be the subject of further study. However, it is clear that the V1 and V2 loops and the V3 loop can affect antibody binding to the CD4 binding site either by direct contact or transmitted conformational effects.

The effects on Fab binding of a series of point mutations in gp120 afford the opportunity to look more closely at recognition differences. The general patterns observed are broadly reminiscent of many CD4 site antibodies and of soluble CD4 itself. Fab b12 is distinguished by its decreased binding to a mutant in which the V1 and V2 loops are deleted. This may or may not be related to the enhanced neutralizing ability of Fab b12. It will be necessary to study a number of variants of Fab b12, which could be produced by chain shuffling or mutation, to answer this question. However, it is clear that the V1 and V2 loops and the V3 loop can affect antibody binding to the CD4 binding site either by direct contact or transmitted conformational effects.

6. Determination of the Relationship Between the
Epitopes Recognized by the Fabs with HIV-1
Antigen Multimeric Complexes

a. Comparison of Fab Epitopes with gp120 and
gp160 Expressed as Multimeric Complexes
on the Surface of COS-1 Cells

5
10
15
20
25
30
35

Given the lack of correlation of Fab neutralization with binding parameters assessed using recombinant gp120, the binding of Fabs b3, b6, and b12 to COS-1 cells expressing the HXBc2 envelope glycoproteins gp160 and gp120 was compared. Fab b3, the poorest neutralizer, Fab b6, also a poor neutralizer, and Fab b12, the most effective neutralizer as determined in Example 5 were used in the assay. The envelope glycoproteins expressed by the COS-1 cells were gp160, the precursor of gp120 and gp41, and the mature gp120. In this assay, different concentrations of Fab were incubated with radiolabeled COS-1 cells which express gp160 and gp120 on their surface. The cells were then washed and lysed. The gp120 and gp160 envelope glycoproteins bound to Fab were precipitated with goat anti-F(ab'), antibody and analyzed by protein gel electrophoresis and shown in Figure 20. Since the amount of HIV-1 envelope glycoprotein expressed on the surface of transfected COS-1 cells is small compared with the amount present intracellularly, after cell lysis, the bound Fab is presented with a large excess of both mature gp120 and gp160 precursor forms. The total amount of envelope glycoproteins precipitated thus provides an indication of the amount of Fab bound to the cell surface. Scanning densitometry profiles were derived from the autoradiographs and are expressed in arbitrary densitometric units.

Although the lack of saturation for Fabs b6 and b3 precludes a precise estimate of affinity, it is clear that Fab b3 exhibits a lower affinity for

the precursor gp160 than either Fab b6 or b12. When the binding of Fab b12 and b6 are compared, several differences are apparent. Assuming that Fab 6 achieves saturation at concentrations slightly higher than 150 $\mu\text{g/ml}$, the estimated affinities of Fab b12 and b6 for the total population of envelope glycoproteins recognized differ only marginally. The most striking difference in the binding of Fab b12 and b6 to the multimeric envelope glycoprotein complex is the preferential detection of gp120 relative to gp160 by Fab b12. Using densitometry to estimate amounts, it is seen from Figure 20 that Fab b12 immunoreacts with an amount of gp120 that is at least about 50 % more than the gp160 present in the immunoreaction admixture. The estimated affinities, based on the Fab concentrations at which half-maximal binding to gp120 is observed, are $3 \times 10^7 \text{ M}^{-1}$ and $<6 \times 10^6 \text{ M}^{-1}$ for Fabs b12 and b6, respectively.

The binding of the Fabs to the multimeric envelope glycoprotein complex on the transfected COS-1 cell surface provides some insights into the observed differences in neutralization potency. The binding of the most potent neutralizing Fab, Fab b12, achieves saturation at roughly 100 $\mu\text{g/ml}$, whereas neither of the less potent neutralizing Fabs achieves saturation even at 150 $\mu\text{g/ml}$. Fab b3 clearly exhibits a lower affinity for the cell surface envelope glycoprotein complex than do the other two Fabs tested, b12 and b6. The most striking difference in the binding of b12 and b6 to the multimeric envelope glycoprotein complex is the preferential precipitation of gp120 relative to gp160 by the bound Fab b12. In addition to these differences in gp120 recognition, it appears that the overall number of cell surface envelope glycoproteins capable of being recognized by the

less neutralizing Fabs is greater than that seen for Fab b12. These differences suggest that Fab b12 may recognize a more limited subset of envelope glycoprotein conformations and that these conformations are better approximated by the mature gp120 glycoprotein in the cell lysates. It is known that the gp160 precursor assumes a greater variety of conformations during the maturation process than does the fully folded gp120 product (Thiriart, et al., J. Immunol., 143:1832-1836 (1989) and Fennie and Lasky, J. Virol., 63:639-646 (1989)). The enhanced neutralization ability of Fab b12 could reflect a higher affinity for a restricted gp120 conformation present in the functionally relevant subset of envelope glycoprotein spikes. Such a functionally relevant group of envelope glycoproteins moieties probably represents a small subset of the total population, consistent with the low infectious fraction associated with HIV-1 and other retroviral virus preparations. One caveat to these observations is that the glycosylation of gp120 expressed as a recombinant protein in baculovirus or on the surface of COS-1 cells is likely to differ and this could affect binding of the Fabs of this invention. However, no difference in the affinity for CD4 binding site antibodies between the two forms of gp120 has been observed previously using a range of antibodies (Moore and Sodroski, unpublished observations). In addition, these studies employed a molecular clone of HIV-1 and its extension to primary isolates will need to be studied further.

Fabs derived from combinatorial libraries may be viewed as "artificial". However, as shown here, the recognition properties of a set of antibodies directed to the CD4 site of gp120 show many features in common with those derived by conventional means. They also show many features

in common with one another suggesting that, with the caveats inherent in the library approach (Barbas et al., J. Molec. Biol., 230:812-823 (1993) and Burton and Barbas, Nature, 359:782-783 (1992)), one individual produces several clearly distinct antibodies directed to a common structural feature, i.e., the CD4 binding site. This is in agreement with observations made on anti-CD4 binding site antibodies using anti-idiotypic antibodies (Chamat et al., J. Immunol., 149:649-654 (1992) and Hariharan et al., J. Virol., 67:953-960 (1993)). One advantage of producing several antibodies is that escape (at least in binding terms) is made more difficult. The only mutations in Table 9 which essentially eliminate the binding of all the antibodies also reduce CD4 binding ability.

The observations presented here have significance for vaccine development. The most effective vaccine may need to induce antibodies to the CD4 binding site with properties similar to those of Fab b12. Given the data above, recombinant gp120 offers no special qualities in this regard. Further, the Fab b12 type of antibody formed only about 10% (4/33 Fabs) of the cloned response of the library donor (Barbas et al., J. Molec. Biol., 230:812-823 (1993)) and has not been described amongst the human antibodies derived by other means suggesting it may be a minor component of typical responses. It is clearly of some interest for vaccine design to define more precisely the structure recognized by Fab b12.

7. Recognition of gp120 from Primary HIV-1 Isolates by b12 IgG1 in Vitro

The ability of the b12 IgG1 to recognize the gp120 molecule from HIV-1 virus from 69 primary isolates was determined in an ELISA assay. Recognition of the primary HIV-1 virus isolate with

b12 IgG1 is indicative of the prevalence of the b12 epitope in the HIV-1 pandemic. To probe the occurrence of the b12 epitope in the HIV-1 pandemic, binding of the b12 IgG1 to gp120 from 69 international isolates belonging to 6 different clades was examined. Virus isolates assayed were obtained from the WHO, HMJFAMM, and NIAID.

Infectious culture supernatants containing virus and free gp120 were treated with 1%(v/v) Nonidet-P40 (NP40) non-ionic detergent to provide a source of gp120 (Moore et al., AIDS, 3:155-160 (1989)). Microplate wells (Immulon II, Dynatech, Ltd.) were first coated with sheep polyclonal antibody D7324. This antibody was raised to the peptide APTKAKRRVVQREKR, derived from the C-terminal 15 amino acids of the clade B IIIB HIV-1 viral isolate. Next, an appropriate volume of inactivated supernatant containing gp120 was diluted with a buffer comprising tris-buffered saline (TBS)/1% (v/v) NP40/10% fetal calf serum (FCS) and a 100 µl aliquot added to the microplate wells for 2 hours at room temperature. Unbound gp120 was removed by washing with TBS, and bound gp120 was detected with CD4-IgG (1 µg/ml) or with b12 IgG1 diluted in a buffer comprising TBS/2%(w/v) nonfat dry milk powder/20%(v/v) sheet serum (TMTSS) essentially as previously described (Moore et al., AIDS, 4:307-310 (1990)) and Moore et al., J. Virol., 68:469-473 (1994)). CD4-IgG is a fusion molecule which consists of CD4 and IgG. The CD4 portion binds to gp120 and the IgG portion provides the means for detection of the CD4-IgG fusion molecule with labeled anti-IgG reagents. Bound antibody was then detected with an appropriate alkaline-phosphatase conjugated anti-IgG, followed by AMPAK (Dako Diagnostics). Absorbance was determined at 492 nm (OD₄₉₂). Each virus was tested against CD4-IgG in triplicate and against b12 IgG1

in duplicate. All OD₄₉₂ values were corrected for non-specific antibody binding in the absence of added gp120 (buffer blank). The mean, blank-corrected OD₄₉₂ values for CD4-IgG and b12 IgG1 were then calculated, and the OD₄₉₂ ratios of b12 IgG1:CD4-IgG were determined. This normalization procedure enables allowance to be made for the different amounts of gp120 captured onto the solid phase via antibody D7324 when comparing antibody reactivity with a panel of viruses. Binding ratios of 0.50 or greater were deemed to represent strong antibody reactivity; ratios from 0.25-0.50 were considered indicative of moderate reactivity; values of <0.25 were designated as representative of essentially negative monoclonal antibody reactivity.

As shown in Figure 22, b12 IgG1 reacts with ≥50% of clades A-D but only 1 of 12 isolates from clade E. Reactivity with clade B isolates from the U.S.A. is approximately 75%.

8. Nucleic Acid Sequence Analysis Comparison Between HIV-1 Specific Monoclonal Antibody Fabs and the Corresponding Derived Amino Acid Residue Sequence

To explore the relationship between neutralizing and weakly or non-neutralizing Fabs, the variable domains of 32 clones expressing human anti-gp120 Fabs, prepared in Example 2 including the 20 listed in Figure 6 for which neutralizing activity was assessed, were sequenced. In addition, the five gp41-specific Fabs were also sequenced.

Nucleic acid sequencing was performed on double-stranded DNA using Sequenase 1.0 (USB, Cleveland, OH) and the appropriate primers hybridizing to sequences in the Cg1 domain (SEQGb : 5' GTCGTTGACCAGGCAGCCCG 3' SEQ ID NO 49) or the Ck

domain (SEQKb : 5' ATAGAAGTTGTTTCAGCAGGCA 3' SEQ ID NO 50). Alternatively sequencing employed single stranded DNA and the T3 primer (5' ATTAACCCCTCACTAAAG 3', SEQ ID NO 51) or one hybridizing to a sequence in the Ck domain (KEF : 5' GAATTCTAAACTAGCTAGTTCG 3' SEQ ID NO 52).

The amino acid residue sequences of the variable heavy and light chains derived from the nucleic acid sequences of the 32 gp120-specific clones are shown respectively in Figures 10 and 11. Groupings are made on the basis of similarities in heavy chain sequences. Dots indicate identity with the first sequence in each section. The SEQ ID NOS are listed to the right of the corresponding derived heavy and light chain (V_H from SEQ ID NO 53-81 and V_L from SEQ ID NO 82-113) amino acid residue sequences in the Figures themselves.

Alignment of derived sequences with one another and with the Genbank database made use of the MacVector suite of programs. For analysis of heavy chain CDR3 sequences as described by Sanz, J. Immunol., 147:1720-1729 (1991), the most 5' nucleotide was considered to be the first nucleotide after codon 95 of the H chain variable region according to Kabat et al, Sequences of Proteins of Immunological Interest, US Dept. of Health and Human Services, Washington, DC (1991). The most 3' nucleotide was assigned to the last unidentified nucleotide before the sequence matched with the published germline JH genes. The CDR3 sequences were analyzed using the DNASTAR software. Sequence comparisons were performed with both the ALIGN and COMPARE programs in order to determine the germline D gene which provided the best homology throughout. In a second step, the SEQCOMP program was used to find sequence identity of at least six nucleotides with either the coding strand or the reverse complement of germline D genes.

The heavy and light chain sequences of the gp41-specific Fabs are shown in Figures 18 and 19, respectively. The amino acid residue sequence of the CDR3 heavy chain exhibits the most variation between the Fabs than any other region of the variable domain.

a. Organization of Antibodies into Groups
According to Heavy Chain Sequence

V_H and V_L domains of 32 gp120 clones were sequenced and the V_H domains compared using MacVector software. This analysis immediately established that a number of the clones, including those selected by panning against different antigens, are closely related to one another. The exception to this is the Fabs selected by panning against the V3 loop peptide which are not related to the Fabs selected by panning against the gp120/160 antigens. Figures 10A and 10B show that the V_H sequences derived from gp120/160 panning can be organized into 7 groups. The broad features apparent from a comparison of amino acid sequences are discussed herein.

The relatedness of sequences within a group varies considerably. For instance, in the group beginning with clone number b8 the amino acid sequences are very similar. Six clones were identical and the remainder showed a maximum of 5 differences from the predominant sequence (the EQ difference due to the 5' primer excluded). Only one clone showed a single difference in the CDR3 region. The average discrepancy over all the sequences in this group from the predominant sequence is 1.1 amino acid residues/ variable domain. This amount corresponds to the order of magnitude of discrepancies which could arise from the PCR. Sequencing of constant domains indicated a PCR error frequency of about 1 base change per

domain.

5 In contrast, in the group headed by clone b3, no two clones were absolutely identical. The average difference from the consensus group sequence is 3.3 residues per sequence and determination for the CDR3 alone is 1.3. Therefore, it seems likely that the heavy chains in this group are somatic variants of one another.

10 The group headed by clone 1 presents a third pattern. Clones b1 and b14 are identical as are clones b2 and B2. However, 23 amino acid differences exist between the two sets of clones. Clones b24 and B30 are approximately equally well differentiated (13-25 differences) from either of these two sets of clones or one another. Still the CDR3 regions are very similar. A number of explanations can be suggested for this pattern: 1) all clones in this group originate from the same germline gene which has undergone extensive somatic mutation, 2) cross-over events have occurred to essentially recombine different germline genes with the same DJ combination, 3) a "convergent evolution" process has led to the selection of different germline genes associated with the same DJ combination.

b. Sequences of the V_H Domains from the gp120 Binders

30 The V_H sequences of the Fabs were organized into the groups defined in Figures 10A and 10B are shown in Figures 11A and 11B. Immediately apparent was the extensive chain promiscuity as evidenced by the pairing of different light chains with the same or a very similar heavy chain with retention of antigen binding capability and indeed, for the most part, antigen affinity as compared with Figures 10A and 10B. This promiscuity can be explored further by

reference to the groups considered above.

5 The clone b8 group, in which the heavy chain members were identical or very similar, also produced 4 light chains which are identical or very similar (less than 3 amino acid differences). Therefore a predominant heavy-light chain combination can be described for this group. One member (clone b8) had the same or very closely related V_L gene but appeared to use a different Jk gene. 10 Two other members (clones B8 and b18) were more distantly related to the major sequence (7-12 differences). Two further clones (b13 and B26) used a Vk gene from a different family, Vk3 compared to Vk1, and therefore were unrelated to 15 the major sequence.

The clone b3 group, suggested to contain somatic variants of a single heavy chain, showed considerable light chain diversity with no two members being closely related to one another. 20 Vk3-Jk2 combinations predominated but Vk3-Jk3 and Vk1-Jk3 combinations also occurred.

On the other hand, in the clone b1 group evidence existed for the heavy chains being more choosy about their light chain partner. Thus, 25 closely related heavy chains appeared to be paired with related light chains. The identical heavy chain pairs (b1 and b14; b2 and B2) had very similar light chains (2 and 4 amino acid differences respectively) whereas the distinct heavy chains (b24 and B30) had distinct light 30 chains which were unrelated to one another or the other group members. The clone 4 group provides another example of this phenomenon in that 4 closely related heavy chains were paired with 3 closely related light chains (a predominant heavy-light chain combination), except for the 35 clone b7 light chain that was distinct.

In summary, the heavy chain (V_H) sequences was organized into 7 groups where each member of a group has an identical or very similar CDR3 region with a limited number of differences elsewhere. When the light chains (V_L) were constrained into the groupings defined by their heavy chain partners, considerable light chain sequence variation was observed. This phenomenon of chain promiscuity has been observed previously and can be appreciated by reference to Figures 11A and 11B. Marked neutralizing ability was confined to two groups of sequences. The first group consisted of Fabs 4, 7, 12 and 21 which have very similar heavy and light chains. The second group consisted of Fabs 13, 8, 18, 22 and 27. Only Fab 13 showed marked neutralizing ability, although the others showed some weaker activity. Interestingly in this group Fab 13 did have a light chain distinct from the other members of the group.

9. Shuffling of the Heavy and Light Chain of a Single Clone Against the Library

To further explore possible functional heavy-light chain combinations, the heavy chain of clone b12 (also referred to as Fab 12 for the corresponding soluble Fab preparation) shown in Figures 10A and 10B was recombined with the original light chain library prepared in Example 2 to construct a new library H12-LCn. In addition, the b12 light chain was recombined with the original heavy chain library to construct a library Hn-L12. These two libraries were taken through 3 rounds of panning against gp120 (IIIB) as described in Example 2b5). The Fabs expressed from the resultant immunoreactant clones were analyzed as described in Example 3 above. Clone b12 was chosen as this Fab neutralized HIV-1 in vitro as shown in Example 3.

To accomplish the preparation of a shuffled library from the Fd gene of clone b12 with the original light chain library, the b12 heavy chain was first subcloned into a tetanus toxoid binding clone expressed in pComb2-3. The light chain library was then cloned into this construction to give a library of 1×10^7 members. The subcloning step was used to avoid contamination with and over-representation of the original light chain. A similar procedure was adopted for shuffling of heavy chains against the light chain from clone b12 to give a library of 3×10^6 members. Cloning and panning procedures were carried out as described above for the original library.

Eleven light chains which recombined with the b12 heavy chain and bound gp120 by panning were randomly chosen for subsequent competition ELISA and sequence analysis. The apparent affinities of these shuffled combinations were similar with an IC_{50} of approximately 10^{-8} to 10^{-9} M. The sequences were organized where a set of 3 were very similar to the original b12 light chain and the other 8 showing many differences from the original with some sub-grouping possible.

The sequences of the light chains which bound to the b12 heavy chain clone are shown in Figures 12A and 12B. The sequences are compared to the sequence for the original light chain from clone b12. The light chains are identified by numbers which do not correspond to the original light chain clones; the assigned numbers of the newly selected clones having new light chains are thus arbitrary. The sequences of these light chains are also listed in the Sequence Listing from SEQ ID NO 114 to 122. Some light chain sequences are identical. In addition to immunoreactivity with gp120, the new Fabs isolated from these shuffled clones were tested in the syncytia assay for neutralization of

HIV-1 infection as described in Example 3. Four shuffled monoclonal Fab antibodies, each having the heavy chain from clone b12, a known HIV-1 neutralizing clone, and new light chains designated L28, L25, L26 and L22, all exhibited approximately 60% neutralization in a syncytia assay with 0.4 $\mu\text{g/ml}$ purified Fab. This effect was equivalent to that obtained with the original clone b12 heavy and light chain pair. Maximum neutralization of approximately 80% was obtained with the H12/L28 and H12/L25 Fabs at 0.7 $\mu\text{g/ml}$ which was equivalent to that seen with the original clone b12 heavy and light pair. The neutralization resulting from the H12/L22 and H12/L26 Fabs plateaued at 60% with Fab concentrations of 0.4 $\mu\text{g/ml}$ up to 1.0 $\mu\text{g/ml}$. Thus, in addition to the gp120 immunoreactive and HIV neutralizing Fabs obtained in the original library prepared as described in Example 2, by shuffling a known neutralizing heavy chain with a library of light chains, new HIV-1 neutralizing Fab monoclonal antibodies have been obtained.

Ten heavy chains which recombined with the b12 light chain were also randomly chosen. One was very similar to the original b12 heavy chain but the others have many differences. Nevertheless, the V-D and D-J junctions were essentially identical indicating the clones had probably arisen from the same rearranged B-cell clone by somatic modification. Competition ELISA failed to reveal any clear difference in affinity between the variants selected from those originally analyzed.

The sequences of the heavy chains which bound to the b12 light chain clone are shown in Figures 13A and 13B. The sequences are compared to the sequence for the original heavy chain from clone b12. The heavy chains are identified by numbers which do not correspond to the original light chain clones; the assigned numbers of the newly selected

clones having new heavy chains are thus arbitrary. The sequences of these light chains are also listed in the Sequence Listing from SEQ ID NO 123 to 132. Some light chain sequences are identical. In addition to immunoreactivity with gp120, the new clones were tested in the syncytia assay for neutralization of HIV-1 infection as described in Example 3. Two shuffled monoclonal Fab antibodies, each having the light chain from clone b12, a known HIV-1 neutralizing clone, and new heavy chains designated H2 and H14, exhibited approximately 40% neutralization in a syncytia assay with 1.0 and 0.5 $\mu\text{g/ml}$ purified Fab, respectively. This effect was equivalent to that obtained with the original clone b12 heavy and light chain pair at a concentration of 2 $\mu\text{g/ml}$. Maximum neutralization of approximately 50% was obtained with the Fab having the new H14 chain at 1.0 $\mu\text{g/ml}$ compared to 80% neutralization with 0.7 $\mu\text{g/ml}$ with the original clone b12 heavy and light pair. Thus, in addition to the gp120 immunoreactive and HIV neutralizing Fabs obtained in the original library prepared as described in Example 2, by shuffling a known neutralizing light chain with a library of heavy chains, new HIV-1 neutralizing Fab monoclonal antibodies have been obtained.

Thus, this shuffling process revealed many more heavy and light chain partners that bound to gp120 that were equal in affinity to those obtained from the original library prepared in Example 2. With this approach, additional HIV-1 neutralizing antibodies can easily be obtained over those present in an original library. The complexity of the clones arising from the heavy chain shuffling also suggests that this approach may be used to map the course of somatic diversification.

Combinatorial libraries randomly recombine heavy and light chains so to what extent antibodies

derived from such libraries represent those produced in a response in vivo can be determined. In principle, a heavy-light chain combination binding antigen could arise fortuitously, i.e.,
5 neither chain is involved in binding antigen in vivo but the combination does bind antigen in vitro.

The available data suggests, however, that heavy chains, from immune libraries, involved in
10 binding antigen tightly in vitro arise from antigen-specific clones in vivo. First, studies have generally failed to identify high-affinity binders in non-immunized IgG libraries. See, Persson et al. Proc. Natl. Acad. Sci., USA,
15 88:2432-2436 (1991) and Marks et al. Eur. J. Immunol., 21:985-991 (1991).

Further, as described above, gp120 binders were not observed in panning a bone marrow IgG library from an HIV seronegative donor against
20 gp120. Second, heavy chains associated with binders from immunized libraries were typically at relatively high frequency in the library indicating they were strongly represented in the mRNA isolated from immunized animals. See, Caton et al., Proc.
25 Natl. Acad. Sci., USA, 87:6450-6454 (1990) and Persson et al., supra. Third, heavy chains from immunized libraries appeared to dictate specificity when recombined with various unrelated light chains as described in Example 10. Fourth, the isolation
30 of intraclonal heavy chain variants as here indicated that an active antibody response was cloned. Thus, the shuffling of a known heavy chain with a light chain binder and vice versa is preferred for use in this invention as new
35 neutralizing Fabs can be obtained beyond those generated in vivo.

Heavy chain promiscuity, i.e., the ability of a heavy chain to pair with different light chains

with retention of antigen affinity, presents serious problems for identifying in vivo light chain partners. This applies not only to the strict definition of partners as having arisen from the same B-cell but also to one which would encompass somatic variants of either partner. The existence of predominant heavy-light chain combinations, particularly involving intraclonal light chain variants, suggests that the light chains concerned are well represented in the library and probably are associated with antigen binding in vivo. However, promiscuity means that, although some combinations probably do occur in vivo, one cannot be certain that one is not shuffling immune partner chains in the recombination. For instance, the occurrence of a virtually identical light chain (b6, B20) in 2 out of 33 clones suggests that it is probably over-represented in the library consistent with an in vivo involvement in antigen-stimulated clones. However, there is no way of knowing whether the in vivo partner of the light chain is the b6 or B20 heavy chain or indeed another heavy chain arising from a stimulated clone.

The light chains arising from the combinatorial library may not be those employed in vivo. Nevertheless it is interesting to note that some heavy chains appear relatively choosy about light chain partner whereas others appear almost indifferent. This observation needs to be tempered by the finding that apparently choosy heavy chains from this analysis will accept diverse light chains with maintenance of antigen binding in a binary plasmid system where pairings are forced as shown below in Example 11 rather than selected in a competitive situation.

Two reports compare heavy-light chain combinations arising from combinatorial libraries

and hybridomas in immunized mice. The library approach begins with mRNA and is therefore probably reflecting plasma cell populations. In contrast, hybridomas are thought to reflect activated but not terminally differentiated B cell populations and EBV transformation to reflect resting B cell populations.

Whatever the arguments about light chain authenticity, the heavy chains of Figures 10A and 10B present many features of interest. The most frequently used heavy chain is of the clone b8 type. It could be argued that this usage simply represents bias in PCR amplification. However, the occurrence of approximately equal numbers of clones in this group amplified by VH1a and VH3a primers argues against this notion. Furthermore, the existence of intraclonal variants in some groups indicates that one is at least sampling different genes from the initial library.

The antibodies cloned here do bear qualitative relationship with the polyclonal antibodies present in the serum of the asymptomatic donor. The titer of anti-gp120 (IIIB) antibodies was approximately 1:3000, with greater than 50% of the reactivity being inhibited by CD4 or a cocktail of Fabs from clones 12, 13 and 14. The titer of anti-gp120 (SF2) antibodies was approximately 1:800. Further, the titer of serum against the short constrained V3 loop peptide was 1:500 and against the full length MN V3 loop peptide was only 1:300. The importance of "anti-CD4 site antibodies" seems general in donors with longer term HIV infection in that the cocktail of Fabs 12, 13 and 14 was able to inhibit binding of a large fraction of serum antibody reactivity with gp120 (IIIB) in 26 of 28 donors tested.

The ability of Fabs to neutralize viruses has been a controversial area. One of the problems has

been that Fabs are classically generated by papain digestion of IgG. If the Fab, as is often the case, shows reduced activity relative to the parent IgG then it may be difficult to rule out IgG contamination in the Fab preparation. Recombinant Fabs, however, as shown herein definitively neutralize virus.

The mechanism of neutralization of HIV-1 appears to neither require virion aggregation nor gp120 cross-linking. In addition, there is no correlation with blocking of the CD4-gp120 interaction to neutralization. The existence of the cloned neutralizing Fabs of this invention should allow the molecular features that confer neutralizing potential to be explored. For instance, in the case of the group of clones containing Fab 13, the unique character of the light chain of that neutralizing clone suggests that chain shuffling experiments in which the 13 light chain was recombined with the other heavy chains in that group, might be revealing. Heavy chains paired with two dissimilar light chains have been shown to retain antigen affinity but exhibit altered fine specificity as shown in Example 11.

The observation here of a large number of Fabs with only a limited number being strongly neutralizing may have important consequences. If the pattern is repeated for whole antibodies then it would seem that much of the gp120 structure may be in a sense a "decoy", i.e., the immune system may invest considerable effort in producing antibodies of high affinity but limited anti-viral function. To exacerbate the situation the ineffective antibodies may bind to gp120 and inhibit the binding of strongly neutralizing antibodies. This has obvious consequences for vaccination which should be primarily designed to elicit neutralizing antibodies of this invention.

10. Shuffling of Selected Heavy and Light Chain
DNA Sequences of a Combinatorial Library in a
Binary Plasmid System

5 A binary system of replicon-compatible
plasmids has been developed to test the potential
for promiscuous recombination of heavy and light
chains within sets of human Fab fragments isolated
from combinatorial antibody libraries. The
efficiency of the system is demonstrated for the
10 combinatorial library of this invention derived
from the bone marrow library of an asymptomatic HIV
donor.

a. Construction of the Binary Plasmid System

15 The binary plasmids pTAC01H and pTC01 for
use in this invention contain the pelB leader
region and multiple cloning sites from Lambda Hc2
and Lambda Lc3, respectively, and the set of
replicon-compatible expression vectors pFL281 and
20 pFL261. Both pFL281 and pFL261 have been described
by Larimer et al., Prot. Eng., 3:227-231 (1990),
the disclosure of which is hereby incorporated by
reference. The nucleotide sequences of pFL261 and
pFL281 are in the EMBL, GenBank and DDBJ Nucleotide
25 Sequence Databases under the accession numbers
M29363 and M68946. The plasmid pFL281 is based on
the plasmid pFL260 also described by Larimer et
al., supra, and having the accession number M29362.
The only distinction between the plasmids pFL260
30 and pFL281 is that pFL281 lacks a 60 bp sequence of
pFL260 between the Eag I site and the Xma III site
resulting in the loss of one of the two BamH I
sites. This deletion is necessary to allow for
cloning of the BamH I Hc2 fragment into the
35 expression vector as described herein.

The replicon-compatible expression vectors
share three common elements: (i) the fl
single-stranded DNA page intergenic IG regions;

(ii) the tightly regulated tac promoter and lac operator; and (iii) an rbs-ATG region with specific cloning sites. The plasmid vectors differ in their antibiotic resistance markers and plasmid replicons: pFL261 carries a gene encoding chloramphenicol acetyltransferase (cat), conferring chloramphenicol resistance, and the p15A replicon; pFL281 carries a gene encoding beta-lactamase (bla), conferring ampicillin resistance, and the ColE1 replicon (ori) from pMB1. The p15A and ColE1 replicons permit the coincident maintenance of both plasmids in the same E. coli host.

The Hc2 and Lc2 vectors prepared in Examples 1a2) and 1a3), respectively, were converted into the plasmid form using standard methods familiar to one of ordinary skill in the art and as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, New York (1989) and subsequently digested with Xho I-Spe I (pHc2) and Sac I-Xba I for (pLc2). The synthetic linkers for insertion into the digested pHc2 and Lc2 plasmids were prepared by American Synthesis. The linkers were inserted to increase the distance between cloning sites so as to increase the effectiveness of the digestions. The 5' and 3' linkers for preparing the double-stranded linker insert into pHc2 were 5' TCGAGGGTCGGTCGGTCTCTAGACGGTCGGTCGGTCA 3' (SEQ ID NO 133) and 5' CTAGTGACCGACCGACCGTCTAGAGACCGACCGACCC 3' (SEQ ID NO 134), respectively. The 5' and 3' linkers for preparing the double-stranded linker insert into pLc2 were 5' CGGTCCGGTCGGTCCTCGAGGGTCGGTCGGTCT 3' (SEQ ID NO 135) and 5' CTAGAGACCGACCGACCCCTCGAGGACCGACCGACCGAGCT 3' (SEQ ID NO 136), respectively. The pairs of linker oligonucleotides were separately ligated to their respective digested, calf intestinal phosphatase-treated vectors.

Subsequently, the multiple cloning sites of pHc2 and pLc2 were transferred into the expression vectors, pFL281 and pFL261, respectively. To accomplish this process, the multiple cloning regions of both Lc2 and Hc2 were separately amplified by PCR as described by Gram et al., Proc. Natl. Acad. Sci., USA, 89:3576-3580 (1992) and as described in Example 2b using Vent Polymerase (New England Biolabs) according to the manufacturer's recommendations. The forward primer, 5' CAAGGAGACAGGATCCATGAAATAC 3' (SEQ ID NO 137) was designed to provide a flush fusion of the pelB leader sequence to the ribosome binding sites of the cloning vectors pFL261 and pFL281 via its internal BamH I site indicated by the underlined nucleotides. The reverse primer 5' AGGGCGAATTGGATCCCGGGCCCCC 3' (SEQ ID NO 138) was designed to anneal downstream of the region of interest in the parent vector of pHc2/pLc2 and create a second BamH I site. The resultant Hc2 and Lc2 PCR amplification products were then digested with BamH I to provide for BamH I overhangs for subsequent ligation into BamH I linearized pFL281 and pFL261 vectors, respectively. The resulting light chain vector containing the Lc2 insert, designated pTC01, was used in this form, whereas the heavy chain vector was further modified with a histidine tail to allow purification of Fab fragments by immobilized metal affinity chromatography as described by Skerra et al., Bio/Technology, 9:273-278 (1991). For this purpose, the synthetic linker oligonucleotides, respectively the 5' and 3' linkers, 5' CTAGTCATCATCATCATTAAGCTAGC 3' (SEQ ID NO 139) and 5' CTAGGCTAGCTTAATGATGATGATGATGA 3' (SEQ ID NO 140) was inserted into the Spe I site, in effect removing the decapeptide tag sequence to generate the heavy chain vector designated as pTAC01H. The

expression of Fab fragment in all subsequent cloning experiments was suppressed by adding 1% (w/v) glucose to all media and plates.

5 b. Construction of Expression Plasmids

For expression of the light chain variable domain, pTC01 prepared above was first digested with Sac I and Xba I; individual light chain inserts were then obtained by separately
10 digesting 22 of the pComb2-3 plasmids prepared and screened as described in Example 2 and listed in Figure 7 that bind to gp120 with the same combination of enzymes and isolating the 0.7 kb fragment using low melting point agarose gel
15 electrophoresis followed by b-agarose digestion. For the chain-shuffling experiments, the following representative members of each of the seven groups shown in Figure 7 were chosen: b11; b6; b4-b12-b7-b21; b3; s8; b1-b14-b24; b13-b22-B26-b8-b18-b27-B8-B35-s4; and one loop
20 peptide-binding clone, p35. The different groups are indicated by semicolon separations while members of the same group are dashed. The resultant isolated light chains were separately
25 ligated into PTC01 overnight at 16°C under standard conditions using a 5:1 molar insert-to-vector ratio to form 21 light chain pTC01 expression vectors. For expression of the heavy chain variable domain, pTAC01H prepared above was first digested with Xho
30 I and Spe I; heavy chain inserts were then obtained by separately PCR amplification reactions of the 20 pComb2-3 plasmids from which light chain inserts were obtained. PCR was used to isolate the heavy chain inserts instead of restriction digestion in
35 order to obtain heavy chain without the cpIII gene anchor sequence in the vector. For the PCR reaction, the respective 5' and 3' primers, 5' CAGGTGCAGCTCGAGCAGTCTGGG 3' (VH1a) (SEQ ID NO 42)

and 5' GCATGTACTAGTTTTGTCACAAGATTTGGG 3' (CG12)
(SEQ ID NO 44) were used to amplify the region
corresponding to the heavy chain as described in
Examples 2a1) and 2a2). The resultant PCR products
5 were purified by low-melting point electrophoresis,
digested with Xho I and Spe I, re-purified, and
separately ligated to the similarly prepared heavy
chain pTAC01H vector using a 1:2 molar vector-to-
insert ratio to form 21 heavy chain pTAC01H
10 expression vectors.

c. Co-transformation of Binary Plasmids

CaCl₂-competent XL1-Blue cells

(Stratagene; recA1, endA1, gyrA96, thi, hsdR17,
15 supE44, relA1, lac, {F' proAB, lacI^q, ZDM15,
Tn10(tet^R)} were prepared and transformed with
approximately 0.5 µg purified DNA of each plasmid
in directed crosses of each of the 20 light chain
vectors with each of the 20 heavy chain vectors.
20 The presence of both plasmids and the episome was
selected for by plating transformants on
triple-antibiotic agar plates (100 µg/ml
carbenicillin, 30 µg/ml chloramphenicol, 10 µg/ml
tetracycline, 32 g/l LB agar) containing 1%
25 glucose.

A binary plasmid system consisting of two
replicon-compatible plasmids was constructed as
shown in 14. The pTAC01H heavy chain vector
schematic is shown in Figure 14A and the pTC01
30 light chain vector schematic is shown in Figure
14B. Both expression vectors feature similar
cloning sites including pel B leader sequences
fused to the ribosome binding sites and the tac
promoters via BamH I sites as shown in Figures 15A
and 15B. The nucleotide sequences of the multiple
35 cloning sites along with the tac promoter, ribosome
binding sites (rbs) and the underlined relevant
restriction sites for the light chain vector,

pTCO1, and heavy chain vector, pTAC01H, are respectively shown in Figure 15A and Figure 15B. The sequences are also listed in the Sequence Listing as described in the Brief Description of the Drawings. The heavy chain vector pTAC01H also contains a (His)₅-tail to allow purification of the recombinant Fab fragments by immobilized metal affinity chromatography. The presence of both plasmids in the same bacterial cell is selected for by the presence of both antibiotics in the media. Expression is partially suppressed during growth by addition of glucose and induced by the addition of IPTG at room temperature. Under these conditions, both plasmids are stable within the cell and support expression of the Fab fragment as assayed by ELISA using goat anti-human kappa and goat anti-human IgG1 antibodies.

d. Preparation of Recombinant Fab Fragments

Bacterial cultures for determination of antigen-binding activity were grown in 96 well-tissue culture plates (Costar #3596). 250 μ l Superbroth [SB had the following ingredients per liter: 10 g 3-(N-morpholino) propanesulfonic acid, 30 g tryptone, 20 g yeast extract at pH 7.0 at 25°C) containing 30 μ g/ml chloramphenicol, 100 μ g/ml carbenicillin, and 1% (w/v) glucose were admixed per well and inoculated with a single double-transformant prepared in Example 11c above. The inoculated plates were then maintained with moderate shaking (200 rpm) on a horizontal shaker for 7-9 hours at 37°C, until the A₅₅₀ was approximately 1-1.5. The cells were collected by centrifugation of the microtiter plate (1,500 X g for 30 minutes at 4°C), the supernatants were discarded, and the cells were resuspended and induced overnight at room temperature in fresh media containing 1 mM IPTG, but no glucose. Cells

were harvested by centrifugation, resuspended in 175 μ l PBS (10 mM sodium phosphate, 160 mM NaCl at pH 7.4 at 25°C) containing 34 μ g/ml phenylmethylsulfonyl fluoride (PMSF) and 1.5% (w/v) streptomycin sulfate, and lysed by 3 freeze-thaw cycles between -80°C and 37°C. The resultant crude extracts were partially cleared by centrifugation as above before analysis by antigen-binding ELISA.

e. Assay and Determination of Relative Affinities

Relative affinities were determined as described in Example 2b6) after coating wells with 0.1 μ g of antigen. The selected antigens included tetanus toxoid and recombinant gp120 (strain IIIB) and gp120 (strain SF2). For each antigen, a negative control extract of XL1-Blue cells co-transformed with pTC01 and pTAC01H was tested to determine whether other components in E. coli had any affinity for the antigens in the assay. Each extract was assayed for BSA-binding activity and BSA-positive clones were considered negative. All possible single-transformants expressing one chain only were prepared as described for the double-transformants and were found to have no affinity for any of the antigens used. Because of the nature of the assay, whether this was due to a lack of binding by the individual chains itself or due to a lack of expression or folding could not be determined.

f. Results of Direct Crosses of Heavy and Light Chains within a Set of gp120/gp160 Binding Antibodies

The Fab fragments derived from the bone marrow of the same asymptomatic HIV donor but panned against gp120 (IIIB), gp160 (IIIB), and

gp120 (SF2), were assigned to one of seven groups based on the amino acid sequences of the CDR3 of their heavy chains as described in Example 9. From the same library, antibodies to the constrained hypervariable v3-loop-like peptide JSISIGPGRAFYTGZC (SEQ ID NO 141) were isolated. For the chain-shuffling experiments, the following representative members of each of the seven groups shown in Figure 7 were chosen: b11; b6; b4-b12-b7-b21; b3; s8; b1-b14-b24; b13-b22-B26-b8-b18-b27-B8-B35-s4; and one loop peptide-binding clone, p35. Clones b4, b7, b12, and b21 showed neutralization activity against HIV when monitoring inhibition of infection by syncytia formation and clones b13, b12, and b4 when monitoring p24 production as shown in Example 3. Light and heavy chains were cloned from the original constructs and cotransformed in all possible binary combinations into XL1-Blue cells as described above.

The results of the complete cross are shown in Figure 16. As is to be expected, identical chains derived from different Fab fragments had similar binding properties e.g., b18HC, b27HC, B8HC, B35HC, s4HC. The crosses of the original heavy chains with the original light chains in each case clearly recapitulated binding activity. Minor differences existed between some heavy chains with identical variable domain sequences, e.g., b4 and b12 (constant domains were not sequenced for any of the constructs). The exception is b8HC, which was identical in its variable domain to b18HC, b27HC, B8HC, B35HC, s4HC, yet shows more cross reactivity. Presumably, this is due to differences in expression levels in the cell or differences in the constant domain sequences. Clear differences existed between heavy chains in their tendency to accept different light chains and still bind antigen, but even the least promiscuous heavy chain

in the set panned against gp120 (IIIB), b1HC, still did so in 43% of its crosses. On the other side of the spectrum, 5 heavy chains, b11HC, b6HC, b12HC, b7HC, and b8HC, crossed productively with all light chains in this set. For the heavy chain crosses examined in detail (all of s4HC, B35HC, B26HC; most of b12HC, b12HC), no significant differences in apparent binding affinity were found between Fab fragments using the same heavy chain but different light chains as shown in Figure 17 where the IC_{50} from competition with soluble gp120 (IIIB) was approximately 10^{-8} M.

Within the original seven groups that were established according to the sequence of the CDR3 of the heavy chains and that are indicated by horizontal and vertical lines in Figure 16, complete promiscuity was present, i.e., heavy and light chains within these CDR3-determined groups were completely promiscuous with each other. However, there was a lack of promiscuity between other groups, e.g., between b1HC-b24HC and b13LC-s4LC. In the analysis of these sequence-based groups, the protein antigen against which the phage display library was panned was not a critical factor. The exception to this case was the cross of p35HC with all light chains; the only cross that bound either to gp120 (SF2 strain) or the original antigen, the loop peptide, was the cross containing the original heavy and light chains.

Unlike the heavy chains, no light chains crossed productively with all heavy chains nor were any distinguishable from the other light chains by unusually low promiscuity.

In the neutralization assays performed as described in Example 3, the directed cross resulting from the pairing of the heavy chain from clone b12 with the light chain from clone b21, was

effective at neutralizing HIV-1.

g. Interantigenic Crosses of Heavy and Light Chains

5 To determine whether conclusions derived from the crosses between high affinity Fab fragments originating from the same library can be extended to unrelated libraries, a non-related gammak-Fab fragment (P3-13) specific for tetanus
10 toxoid from a different donor was chosen for a new set of crosses [clone 3 in Persson et al., Proc. Natl. Acad. Sci., USA, 88:2432-2436 (1991)]. Extracts were probed with tetanus toxoid or with gp120 (IIIB). The data confirm the results from
15 the gp120 cross experiment in that the binding activity towards the antigen was determined by the heavy chain. The heavy chain of clone P3-13 paired with the light chains b4, b12, b21, and b14 to yield an Fab fragment with an affinity towards
20 tetanus toxoid; the light chain of P3-13 paired with the heavy chains of b3, b6, b11, and b14 to yield an Fab fragment with an affinity towards gp120 (IIIB). None of the light chains originating from the gp120 binders was able to confer gp120
25 specificity in combination with the P3-13 heavy chain.

 Similarly, the P3-13 light chain was unable to generate tetanus toxoid specificity in combination with any of the heavy chains originating from the
30 gp120 binders, confirming the dominance of the heavy chain in the antibody-antigen interaction. Interestingly, all three light chains that showed a strong signal against tetanus toxoid (b4, b12, b21) were members of the same group when sorted by the
35 CDR3's of their original heavy chains. As might be expected from crosses between unrelated libraries, not only was there a lower degree of promiscuity, i.e., chains paired productively with far fewer

complementary chains, but the range of apparent affinity constants determined by competition ELISA was much broader (6.3×10^6 - 6.3×10^8 M). The replacement of the original P3-13 light chain in the P3-13 Fab fragment with another light chain lowered the affinity of the Fab towards tetanus toxoid 10 to 100-fold (from 6.3×10^8 M to 6.3×10^6 M). In the crosses of the light chain of P3-13 with all the heavy chains of the HIV pannings, the productive crosses had similar affinities to gp120 (IIIB) (2.5×10^7 - 6.3×10^7 M), with the exception of b14HC/P3-13LC, whose signal was too weak for a definite determination of the apparent binding constant. These affinities were approximately five-fold lower than those of the gp120-heavy chains with their original light chains.

Thus, the results show that chain shuffling is yet another maneuver allowed in vitro but not in vivo which can be expected to help extend antibody diversity beyond that of Nature. The overriding feature of the binary system of this invention is its ability to create large numbers (several hundred) of directed crosses between characterized light and heavy chains without the need for recloning individual chains for each cross after the initial vector construction. When used in combination with the phage-display method and biological assays, it allows the rapid analysis of the most interesting subset of the pool of antigen-binding clones by chain shuffling, with the aim of finding biologically or chemically active antibodies. For the set of antigens studied here, most heavy chains recombined with a number of light chains to yield an antigen-binding Fab fragment.

These results have important implications for the diversity of combinatorial antibody libraries. While it is not possible to predict reliably the original in vivo combinations of light and heavy

chains due to the surprising promiscuity of individual chains, recombinant antibody libraries take advantage of the fact that even distantly related Fabs against the same antigen can recombine in vitro to give chain combinations not found in vivo. In fact, after the identification of a certain number of antibodies that have been shown to possess some biological or chemical activity, it may be better to shuffle their individual chains in a directed fashion than to continue sampling randomly from the same pool of binders. By extension, the promiscuity observed in this system indicates that in libraries constructed using degenerate, chemically synthesized oligonucleotides, there should be considerable flexibility in which separate synthetic heavy chains can pair with separate synthetic light chains to generate separate antigen-binding Fab fragments. The diversity of combinatorial libraries coupled with chain-shuffling should allow wide exploration of three dimensional space thereby solving the problem of how to approximate molecules in the ternary complex of antibody, substrate and cofactor.

11. Deposit of Materials

The following cell lines have been deposited on September 30, 1992, with the American Type Culture Collection (ATCC), 1301 Parklawn Drive, Rockville, MD, USA:

<u>Cell Line</u>	<u>ATCC Accession No.</u>
<u>E. coli</u> MT11	ATCC 69078
<u>E. coli</u> MT12	ATCC 69079
<u>E. coli</u> MT13	ATCC 69080

The deposits listed above, MT11, MT12 and MT13 are bacterial cells (E. coli) containing the

expression vector pComb2-3 for the respective
expression of the Fabs designated b11 (clone b11),
b12 (clone b12), and b13 (clone b13) prepared in
Example 2b. The sequences of the heavy and light
chain variable domains are listed in Figures 10A
and 10B and 11A and 11B, respectively. This
deposit was made with the ATCC under the provisions
of the Budapest Treaty on the International
Recognition of the Deposit of Microorganisms for
the Purpose of Patent Procedure and the Regulations
thereunder (Budapest Treaty). This assures
maintenance of a viable culture for 30 years from
the date of deposit. The organisms will be made
available by ATCC under the terms of the Budapest
Treaty which assures permanent and unrestricted
availability of the progeny of the culture to the
public upon issuance of the pertinent U.S. patent
or upon laying open to the public of any U.S. or
foreign patent application, whichever comes first,
and assures availability of the progeny to one
determined by the U.S. Commissioner of Patents and
Trademarks to be entitled thereto according to 35
U.S.C. §122 and the Commissioner's rules pursuant
thereto (including 37 CFR §1.14 with particular
reference to 886 OG 638). The assignee of the
present application has agreed that if the culture
deposit should die or be lost or destroyed when
cultivated under suitable conditions, it will be
promptly replaced on notification with a viable
specimen of the same culture. Availability of the
deposited strain is not to be construed as a
license to practice the invention in contravention
of the rights granted under the authority of any
government in accordance with its patent laws.

35

The foregoing written specification is
considered to be sufficient to enable one skilled
in the art to practice the invention. The present

invention is not to be limited in scope by the cell lines deposited, since the deposited embodiment is intended as a single illustration of one aspect of the invention and any cell lines that are functionally equivalent are within the scope of this invention. The deposit of material does not constitute an admission that the written description herein contained is inadequate to enable the practice of any aspect of the invention, including the best mode thereof, nor is it to be construed as limiting the scope of the claims to the specific illustration that it represents. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and fall within the scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: THE SCRIPPS RESEARCH INSTITUTE
(B) STREET: 10666 North Torrey Pines Road
(C) CITY: La Jolla
(D) STATE: CA
(E) COUNTRY: USA
(F) POSTAL CODE (ZIP): 92037
(G) TELEPHONE: 619-554-2937
(H) TELEFAX: 619-554-6312

(ii) TITLE OF INVENTION: HUMAN NEUTRALIZING MONOCLONAL ANTIBODIES
TO HUMAN IMMUNODEFICIENCY VIRUS

(iii) NUMBER OF SEQUENCES: 170

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(v) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: PCT/US 95/
(B) FILING DATE: 11-JUL-1995

(vi) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/276,852
(B) FILING DATE: 18-JUL-1994

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 173 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GGCCGCAAAT TCTATTTCAA GGAGACAGTC ATAATGAAAT ACCTATTGCC TACGGCAGCC 60
GCTGGATTGT TATTACTCGC TGCCCAACCA GCCATGGCCC AGGTGAAACT GCTCGAGATT 120
TCTAGACTAG TTACCCGTAC GACGTTCCGG ACTACGGTTC TTAATAGAAT TCG 173

(2) INFORMATION FOR SEQ ID NO:2:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 173 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(11) MOLECULE TYPE: DNA (genomic)

(111) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:2:

TCGACGAATT CTATTAAGAA CCGTACTCCG GAACGTCGTA CGGGTAACTA GTCTAGAAAT 60
CTCGAGCAGT TTCACCTGGG CCATGGCTGG TTGGGCAGCG AGTAATAACA ATCCAGCGGC 120
TGCCGTAGCC AATAGGTATT TCATTATGAC TGTCTCCTTG AAATAGAATT TGC 173

(2) INFORMATION FOR SEQ ID NO:3:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 131 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(11) MOLECULE TYPE: DNA (genomic)

(111) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TGAATTCTAA ACTAGTCGCC AAGGAGACAG TCATAATGAA ATACCTATTG CCTACGGCAG 60
CCGCTGGATT GTTATTACTC GCTGCCCAAC CAGCCATGGC CGAGCTCGTC AGTTCTAGAG 120
TTAAGCGGCC G 131

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 139 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TCGACGGCCG CTAACTCTA GAACTGACGA GCTCGGCCAT GGCTGGTTGG GCAGCGAGTA 60
ATAACAATCC AGCGGCTGCC GTAGGCAATA GGTATTTTCAT TATGACTGTC TCCTTGGCGA 120
CTAGTTTAGA ATTCAAGCT 139

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Ser
1 5 10

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 26 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Ala
1 5 10 15

Ala Gln Pro Ala Met Ala Gln Val Lys Leu
 20 25

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 23 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met Lys Tyr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Ala
1 5 10 15

Ala Gln Pro Ala Met Ala Glu
 20

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 198 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TGTTGACAAT TAATCATCGG CTCGTATAAT GTGTGGAATT GTGAGCGGAT AACAAATTCA	60
CACAGGAGGA AGGATCCATG AAATACCTAT TGCCTACGGC AGCCGCTGGA TTGTIATTAC	120
TCGCTGCCCCA ACCAGCCATG GCCGAGCTCG GTCGGTCGGT CCTCGAGGGT CCGTCGGTCT	180
CTAGACTTAA GCGGCCCG	198

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 198 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GCGGCCGCTT AACTCTAGAG ACCGACCGAC CCTCGAGGAC CGACCGACCG AGCTCGGCCA	60
TGGCTGGTTG GGCAGCGAGT AATAACAATC CAGCGGCTGC CGTAGGCAAT AGGTATTTCA	120
TGGATCCTTC CTCCTGTGTG AAATTGTTAT CCGCTCACAA TTCCACACAT TATACGAGCC	180
GATGATTAAT TGTCAACA	198

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Met Lys Thr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Ala
 1 5 10 15
 Ala Gln Pro Ala Met Ala Glu Leu
 20

(2) INFORMATION FOR SEQ ID NO:11:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 220 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: circular

(1i) MOLECULE TYPE: DNA (genomic)

(1ii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:11:

TGTTGACAAT TAATCATCGG CTCGTATAAT GTGTGGAATT GTGAGCGGAT AACAAATTCA 60
 CACAGGAGGA AGGATCCATG AAATACCTAT TGCCTACGGC AGCCGCTGGA TTGTTATTAC 120
 TCGCTGCCCA ACCAGCCATG GCCGAGGTGA AACTGCTCGA GGGTCGGTCG GTCTCTAGAC 180
 GGTCCGTCGG TCACTAGTCA TCATCATCAT CATTAAAGCTA 220

(2) INFORMATION FOR SEQ ID NO:12:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 220 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: circular

(1i) MOLECULE TYPE: DNA (genomic)

(1ii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TAGCTTAATG ATGATGATGA TGACTAGTGA CCGACCGACC GTCTAGAGAC CGACCGACCC 60
TCGAGCAGTT TCACCTGGGC CATGGCTGGT TGGGCAGCGA GTAATAACAA TCCAGCGGCT 120
GCCGTAGGCA ATAGGTATTT CATGGATCCT TCCTCCTGTG TGAAATTGTT ATCCGCTCAC 180
AATCCACAC ATTATACGAG CCGATGATTA ATTGTCAACA 220

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Met Lys Thr Leu Leu Pro Thr Ala Ala Ala Gly Leu Leu Leu Ala
1 5 10 15

Ala Gln Pro Ala Met Ala Gln Val Lys Leu Leu Glu
20 25

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: C-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Thr Ser His His His His His
1 5

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGCCGCAAAT TCTATTTC AA GGAGACAGTC AT

32

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

AATGAAATAC CTATTGCCTA CGGCAGCCGC TGGATT

36

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CTTATTACTC GCTGCCCAAC CAGCCATGGC CC

32

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CAGTTTCACC TGGGCCATGG CTGGTTGGG

29

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 40 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CAGCGAGTAA TAACAATCCA GCGGCTGCCG TAGGCAATAG

40

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 38 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GTATTCATT ATGACTGTCT CCTTGAAATA GAATTTCG

38

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

AGGTGAAACT GCTCGAGATT TCTAGACTAG TTACCCGTAC

40

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 38 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

CGGAACGTCG TACGGGTAAC TAGTCTAGAA ATCTCGAG

38

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 33 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

GACGTTCCGG ACTACGGTTC TTAATAGAAT TCG

33

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 28 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TCGACGAATT CTATTAAGAA CCGTAGTC

28

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 34 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TGAATTCTAA ACTAGTCGCC AAGGAGACAG TCAT

34

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

AATGAAATAC CTATTGCCTA CGGCAGCCGC TGGATT

36

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

GTTATTACTC GCTGCCCAAC CAGCCATGGC C

31

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GAGCTCGTCA GTTCTAGAGT TAAGCGGCCG

30

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 48 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GTATTTTCATT ATGACTGTCT CCTTGGCGAC TAGTTTAGAA TTCAAGCT

48

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CAGCGAGTAA TAACAATCCA GCGGCTGCCG TAGGCAATAG

40

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 27 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

TGACGAGCTC GGCCATGGCT GGTGGG

27

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 24 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

TCGACGGCCG CTAACTCTA GAAC

24

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 666 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```

CCATTCGTTT GTGAATATCA AGGCCAAGGC CAATCGTCTG ACCTGCCTCA ACCTCCTGTC      60
AATGCTGGCG GCGGCTCTGG TGGTGGTTCT GGTGGCGGCT CTGAGGGTGG TGGCTCTGAG      120
GGTGGCGGTT CTGAGGGTGG CGGCTCTGAG GGAGGCGGTT CCGGTGGTGG CTCTGGTTCC      180
GGTGATTTTG ATTATGAAAA GATGGCAAAC GCTAATAAGG GGGCTATGAC CGAAAATGCC      240
GATGAAAACG CGCTACAGTC TGACGCTAAA GGCAAACTTG ATTCTGTCCG TACTGATTAC      300
GGTGCTGCTA TCGATGTTT CATTGGTGAC GTTCCGGCC TTGCTAATGG TAATGGTGCT      360
ACTGGTGATT TTGCTGGCTC TAATCCCAA ATGGCTCAAG TCGGTGACGG TGATAATTCA      420
CCTTTAATGA ATAATTCCG TCAATATTTA CCTCCCTCC CTCAATCGGT TGAATGTCCG      480
CCTTTTGTCT TTAGCGCTGG TAAACCATAT GAATTTTCTA TTGATTGTGA CAAAATAAAC      540
TTATTCGGTG TCTTTGCGTT TCTTTTATAT GTTGCCACCT TTATGTATGT ATTTTCTACG      600
TTTGCTAACA TACTGCGTAA TAAGGAGTCT TAATCATGCC AGTTCTTTTG GGTATTCGGT      660
TATTAT

```

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 211 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: unknown

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

```

Pro Phe Val Cys Glu Tyr Gln Gly Gln Gly Gln Ser Ser Asp Leu Pro
1           5           10           15
Gln Pro Pro Val Asn Ala Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly
20           25           30

```

Gly Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly
 35 40 45
 Ser Glu Gly Gly Gly Ser Gly Gly Gly Ser Gly Ser Gly Asp Phe Asp
 50 55 60
 Tyr Glu Lys Met Ala Asn Ala Asn Lys Gly Ala Met Thr Glu Asn Ala
 65 70 75 80
 Asp Glu Asn Ala Leu Gln Ser Asp Ala Lys Gly Lys Leu Asp Ser Val
 85 90 95
 Ala Thr Asp Tyr Gly Ala Ala Ile Asp Gly Phe Ile Gly Asp Val Ser
 100 105 110
 Gly Leu Ala Asn Gly Asn Gly Ala Thr Gly Asp Phe Ala Gly Ser Asn
 115 120 125
 Ser Gln Met Ala Gln Val Gly Asp Gly Asp Asn Ser Pro Leu Met Asn
 130 135 140
 Asn Phe Arg Gln Tyr Leu Pro Ser Leu Pro Gln Ser Val Glu Cys Arg
 145 150 155 160
 Pro Phe Val Phe Ser Ala Gly Lys Pro Tyr Glu Phe Ser Ile Asp Cys
 165 170 175
 Asp Lys Ile Asn Leu Phe Arg Gly Val Phe Ala Phe Leu Leu Tyr Val
 180 185 190
 Ala Thr Phe Met Tyr Val Phe Ser Thr Phe Ala Asn Ile Leu Arg Asn
 195 200 205
 Lys Glu Ser
 210

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 48 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

GAGACGACTA GTGGTGGCGG TGGCTCTCCA TTCGTTTG TG AATATCAA

48

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 40 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

TTACTAGCTA GCATAATAAC GGAATACCCA AAAGAACTGG

40

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

TATGCTAGCT AGTAACACGA CAGGTTTCCC GACTGG

36

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 27 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

ACCGAGCTCG AATTCGTAAT CATGGTC

27

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

AGCTGTTGAA TTCGTGAAAT TGTATCCGC T

31

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 708 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GAGACCACTA GTGGTGGCGG TGGCTCTCCA TTCGTTTGTG AATATCAAGG CCAAGGCCAA

60

TCGTCGACC TGCCTCAACC TCCTGTCAAT GCTGGCGGCG GCTCTGGTGG TGGTTCTGGT 120
GGCGGCTCTG AGGGTGGTGG CTCTGAGGGT GCGGGTTCTG AGGGTGGCGG CTCTGAGGGA 180
GGCGGTTCGG GTGGTGGCTC TGGTTCGGGT GATTTTGATT ATGAAAAGAT GGCAAACGCT 240
AATAAGGGGG CTATGACCGA AAATGCCGAT GAAAACGCCG TACAGTCTGA CGCTAAAGGC 300
AAACTTGATT CTGTCGCTAC TGATTACGGT GCTGCTATCG ATGGTTTCAT TGGTGACGTT 360
TCCGGCCTTG CTAATGGTAA TGGTGCTACT GGTGATTTTG CTGGCTCTAA TTCCCAAATG 420
GCTCAAGTCG GTGACGGTGA TAATTCACCT TTAATGAATA ATTTCCGTCA ATATTTACCT 480
TCCCTCCCTC AATCGGTTGA ATCTCGCCCT TTTGTCTTTA GCGCTGGTAA ACCATATGAA 540
TTTTCTATTG ATTGTGACAA AATAAACTTA TTCCGTGGTG TCTTTGCGTT TCTTTTATAT 600
GTTGCCACCT TTATGTATGT ATTTTCTACG TTTGCTAACA TACTGCGTAA TAAGGAGTCT 660
TAATCATGCC AGTTCITTTG GGTATTCGGT TATTATGCTA GCTAGTAA 708

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 201 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

TATGCTAGCT AGTAACACGA CAGGTTTCCC GACTGGAAAG CGGGCAGTGA GCGCAACGCA 60
ATTAATGTGA GTTAGCTCAC TCATTAGGCA CCCAGGCTT TACACTTTAT GCTTCCGGCT 120
CGTATGTTGT GTGGAATTGT GAGCGGATAA CAATTTCACA CAGGAAACAG CTATGACCAT 180
GATTACGAAT TCGAGCTCGG T 201

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

CAGGTGCAGC TCGAGCAGTC TGGG

24

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

GAGGTGCAGC TCGAGGAGTC TGGG

24

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

GCATGTACTA GTTTGTCAC AAGATTGGG

30

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

GACATCGAGC TCACCCAGTC TCCA

24

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

GAAATTGAGC TCACGCAGTC TCCA

24

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 53 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

GCGCCGTCTA GAACTAACAC TCTCCCCTGT TGAAGCTCTT TGTGACGGGC AAG

53

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 12 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Ser Ile Ser Gly Pro Gly Arg Ala Phe Tyr Thr Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

GTCGTTGACC AGGCAGCCCA G

21

(2) INFORMATION FOR SEQ ID NO:50:

- (1) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

ATAGAAGTTG TTCAGCAGGC A

21

(2) INFORMATION FOR SEQ ID NO:51:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 17 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

ATTAACCCTC ACTAAAG

17

(2) INFORMATION FOR SEQ ID NO:52:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

GAATTCTAAA CTAGCTAGTT CG

22

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 128 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Leu Glu Glu Ser Gly Thr Glu Phe Lys Pro Pro Gly Ser Ser Val Lys
1 5 10 15

Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Gly Asp Tyr Ala Ser Asn
20 25 30

Tyr Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Tyr
35 40 45

Ile Gly Gly Ile Thr Pro Thr Ser Gly Ser Ala Asp Tyr Ala Gln Lys
50 55 60

Phe Gln Gly Arg Val Thr Ile Ser Ala Asp Arg Phe Thr Pro Ile Leu
65 70 75 80

Tyr Met Glu Leu Arg Ser Leu Arg Ile Glu Asp Thr Ala Ile Tyr Tyr
85 90 95

Cys Ala Arg Glu Arg Arg Glu Arg Gly Trp Asn Pro Arg Ala Leu Arg
100 105 110

Gly Ala Leu Asp Phe Trp Gly Gln Gly Thr Arg Val Phe Val Ser Pro
115 120 125

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 124 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Leu Glu Glu Ser Gly Ala Ala Val Gln Lys Pro Gly Ser Ser Val Arg
 1 5 10 15
 Val Ser Cys Gln Ala Ser Gly Gly Thr Phe Asp Asn Phe Ala Ser Asn
 20 25 30
 Tyr Ala Val Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp
 35 40 45
 Met Gly Gly Ile Thr Pro Thr Ser Gly Thr Ala Thr Tyr Ser Gln Lys
 50 55 60
 Phe Gln Gly Arg Val Thr Ile Ser Ala Ala Pro Leu Thr Pro Ile Ile
 65 70 75 80
 Tyr Met Glu Leu Arg Ser Leu Arg Asp Asp Asp Thr Ala Val Tyr Tyr
 85 90 95
 Cys Ala Arg Glu Arg Arg Glu Arg Gly Trp Asn Pro Arg Ala Leu Val
 100 105 110
 Gly Ala Leu Asp Val Trp Gly Gln Gly Thr Thr Val
 115 120

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 128 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Leu Glu Glu Ser Gly Thr Glu Phe Lys Pro Pro Gly Ser Ser Val Lys
 1 5 10 15
 Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Gly Asp Tyr Ala Ser Asn
 20 25 30
 Tyr Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Tyr
 35 40 45
 Ile Gly Gly Ile Thr Pro Thr Ser Gly Ser Ala Asp Tyr Ala Gln Lys
 50 55 60

Phe Gln Gly Arg Val Thr Ile Ser Ala Asp Arg Phe Thr Pro Ile Leu
 65 70 75 80
 Tyr Met Glu Leu Arg Ser Leu Arg Ile Glu Asp Thr Ala Ile Tyr Tyr
 85 90 95
 Cys Ala Arg Glu Arg Arg Glu Arg Gly Trp Asn Pro Arg Ala Leu Arg
 100 105 110
 Gly Ala Leu Asp Phe Trp Gly Gln Gly Thr Arg Val Phe Val Ser Pro
 115 120 125

(2) INFORMATION FOR SEQ ID NO:56:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 128 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(11) MOLECULE TYPE: protein

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Leu Glu Glu Ser Gly Ala Glu Val Lys Lys Pro Gly Ser Ser Val Lys
 1 5 10 15
 Val Ser Cys Lys Ala Ser Gly Gly Ile Phe Ser Asp Phe Ala Ser Asn
 20 25 30
 Tyr Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Tyr
 35 40 45
 Met Gly Gly Ile Thr Pro Thr Ser Gly Ser Ala Asp Tyr Ala Gln Lys
 50 55 60
 Phe Gln Gly Arg Val Thr Ile Ser Ala Asp Ala Ala Thr Pro Arg Val
 65 70 75 80
 Tyr Met Glu Leu Arg Ile Leu Arg Ser Glu Asp Thr Ala Val Tyr Phe
 85 90 95
 Cys Ala Arg Glu Arg Arg Glu Arg Gly Trp Asn Pro Arg Ala Leu Arg
 100 105 110
 Gly Ala Leu Glu Val Trp Gly Gln Gly Thr Thr Val Ile Val Ser Pro
 115 120 125

(2) INFORMATION FOR SEQ ID NO:57:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 128 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

```

Leu Glu Glu Ser Gly Ala Ala Val Gln Lys Pro Gly Ser Ser Val Arg
1           5           10           15
Val Ser Cys Gln Ala Ser Gly Gly Thr Phe Asp Asn Phe Ala Ser Asn
20           25           30
Tyr Ala Val Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp
35           40           45
Met Gly Gly Ile Thr Pro Thr Ser Gly Thr Ala Thr Tyr Ser Gln Lys
50           55           60
Phe Gln Gly Arg Val Thr Ile Ser Ala Ala Pro Leu Thr Pro Ile Ile
65           70           75           80
Tyr Met Glu Leu Arg Ser Leu Arg Asp Asp Asp Thr Ala Val Tyr Tyr
85           90           95
Cys Ala Arg Glu Arg Arg Glu Arg Gly Trp Asn Pro Arg Ala Leu Val
100          105          110
Gly Ala Leu Asp Val Trp Gly Gln Gly Thr Thr Val Ile Val Ser Ser
115          120          125

```

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 128 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

```

Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ser Ser Val Lys
1           5           10           15

```

Val Ser Cys Lys Thr Ser Gly Gly Thr Phe Ser Asp Tyr Ala Ser Asn
 20 25 30
 His Ala Ile Ser Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Tyr
 35 40 45
 Met Gly Gly Ile Thr Pro Thr Ser Gly Thr Ala Asp Tyr Ala Gln Lys
 50 55 60
 Phe Gln Ala Arg Val Thr Ile Ser Ala His Glu Phe Thr Pro Ile Val
 65 70 75 80
 Tyr Met Glu Leu Arg Ser Leu Arg Ser Asp Gln His Ala Thr Tyr Tyr
 85 90 95
 Cys Ala Thr Glu Arg Arg Glu Arg Gly Trp Asn Pro Arg Ala Leu Arg
 100 105 110
 Gly Ala Leu Asp Ile Trp Gly Gln Gly Thr Thr Val Ile Val Ser Ser
 115 120 125

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 128 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

Leu Glu Glu Ser Gly Gly Arg Leu Val Lys Pro Gly Gly Ser Leu Arg
 1 5 10 15
 Leu Ser Cys Glu Gly Ser Gly Phe Thr Phe Thr Asn Ala Trp Met Thr
 20 25 30
 Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Val Ala Ser Ile
 35 40 45
 Lys Ser Lys Phe Asp Gly Gly Ser Pro His Tyr Ala Ala Pro Val Glu
 50 55 60
 Gly Arg Phe Ser Ile Ser Arg Asn Asp Leu Glu Asp Lys Met Phe Leu
 65 70 75 80
 Glu Met Ser Gly Leu Lys Ala Glu Asp Thr Gly Val Tyr Tyr Cys Ala
 85 90 95

Thr Lys Tyr Pro Arg Tyr Ser Asp Met Val Thr Gly Val Arg Asn His
 100 105 110

Phe Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Ile Val Ser Ser
 115 120 125

(2) INFORMATION FOR SEQ ID NO:60:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 128 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(11) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

Leu Glu Gln Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Arg
 1 5 10 15

Leu Ser Cys Glu Gly Ser Gly Phe Thr Phe Thr Asn Ala Trp Met Thr
 20 25 30

Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Val Ala Ser Ile
 35 40 45

Lys Ser Lys Phe Asp Gly Gly Ser Pro His Tyr Ala Ala Pro Val Glu
 50 55 60

Gly Arg Phe Thr Ile Ser Arg Asn Asp Leu Glu Asp Lys Leu Phe Leu
 65 70 75 80

Glu Met Ser Gly Leu Lys Ala Glu Asp Thr Gly Val Tyr Tyr Cys Ala
 85 90 95

Thr Lys Tyr Pro Arg Tyr Phe Asp Met Met Ala Gly Val Arg Asn His
 100 105 110

Phe Tyr Met Asp Val Trp Gly Thr Gly Thr Thr Val Ile Val Ser Ser
 115 120 125

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 128 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(11) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

```

Leu Glu Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Arg
 1             5             10             15
Leu Ser Cys Glu Gly Ser Gly Phe Thr Phe Thr Asn Ala Trp Met Thr
          20             25             30
Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Val Ala Ser Ile
          35             40             45
Lys Ser Lys Phe Asp Gly Gly Ser Pro His Tyr Ala Ala Pro Val Glu
          50             55             60
Gly Arg Phe Thr Ile Ser Arg Asn Asp Leu Glu Asp Lys Leu Phe Leu
 65             70             75             80
Glu Met Ser Gly Leu Lys Ala Glu Asp Thr Gly Val Tyr Tyr Cys Ala
          85             90             95
Thr Lys Tyr Pro Arg Tyr Ser Asp Met Met Ala Gly Val Arg Asn His
          100            105            110
Leu Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Ile Val Ser Ser
          115            120            125

```

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 128 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(11) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

```

Leu Glu Glu Ser Gly Gly Arg Leu Val Lys Pro Gly Gly Ser Leu Arg
 1             5             10             15
Leu Ser Cys Glu Ala Ser Gly Phe Thr Phe Thr Asn Ser Trp Met Thr
          20             25             30
Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Val Ala Ser Ile
          35             40             45

```

Lys Arg Lys Phe Asp Gly Gly Ser Pro His Tyr Ala Ala Pro Val Glu
 50 55 60
 Gly Arg Phe Ser Ile Ser Arg Asn Asp Leu Glu Asp Lys Met Phe Leu
 65 70 75 80
 Glu Met Ser Gly Leu Lys Ala Glu Asp Thr Gly Val Tyr Tyr Cys Ala
 85 90 95
 Thr Lys Tyr Pro Arg Tyr Ser Asp Met Met Thr Gly Val Arg Asn His
 100 105 110
 Phe Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Ile Val Ser Ser
 115 120 125

(2) INFORMATION FOR SEQ ID NO:63:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 128 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

Leu Glu Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Arg
 1 5 10 15
 Leu Ser Cys Glu Ser Ser Gly Phe Thr Phe Thr Asn Ala Trp Met Thr
 20 25 30
 Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Val Ala Ser Ile
 35 40 45
 Lys Ser Lys Phe Asp Gly Gly Ser Pro His Tyr Ala Ala Pro Val Glu
 50 55 60
 Gly Arg Phe Thr Ile Ser Arg Asn Asp Leu Glu Asp Lys Leu Phe Leu
 65 70 75 80
 Glu Met Ser Gly Leu Lys Ala Glu Asp Thr Gly Val Tyr Tyr Cys Ala
 85 90 95
 Thr Lys Tyr Pro Arg Tyr Ser Asp Met Met Ala Gly Val Arg Asn His
 100 105 110
 Phe Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Ile Val Ser Ser
 115 120 125

(2) INFORMATION FOR SEQ ID NO:64:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 128 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

Leu Glu Glu Ser Gly Gly Arg Leu Val Lys Pro Gly Gly Ser Leu Arg
1 5 10 15
Leu Ser Cys Glu Gly Ser Gly Phe Thr Phe Thr Asn Ala Trp Met Thr
20 25 30
Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Val Ala Ser Ile
35 40 45
Lys Ser Lys Phe Asp Gly Gly Ser Pro His Tyr Ala Ala Pro Val Glu
50 55 60
Gly Arg Phe Ser Ile Ser Arg Asn Asp Leu Glu Asp Lys Met Phe Leu
65 70 75 80
Glu Met Ser Gly Leu Lys Ala Glu Asp Thr Gly Val Tyr Tyr Cys Ala
85 90 95
Thr Lys Tyr Pro Arg Tyr Ser Asp Met Met Thr Gly Val Arg Asn His
100 105 110
Phe Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Ile Val Ser Ser
115 120 125

(2) INFORMATION FOR SEQ ID NO:65:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 128 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

Leu Glu Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Arg
 1 5 10 15
 Leu Ser Cys Ala Gly Ser Gly Phe Thr Phe Thr Asn Ala Trp Met Thr
 20 25 30
 Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Val Ala Ser Ile
 35 40 45
 Lys Ser Lys Phe Asp Gly Gly Ser Ser His Tyr Pro Gly Pro Val Glu
 50 55 60
 Gly Arg Phe Thr Ile Ser Arg Asn Tyr Ile Glu Asp Lys Leu Phe Leu
 65 70 75 80
 Glu Met Ser Gly Leu Lys Ala Glu Asp Thr Gly Val Tyr Tyr Cys Ala
 85 90 95
 Thr Lys Tyr Pro Arg Tyr Tyr Asp Met Met Arg Gly Val Arg Asn His
 100 105 110
 Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Ile Val Ser Ser
 115 120 125

(2) INFORMATION FOR SEQ ID NO:66:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 124 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys
 1 5 10 15
 Val Ser Cys Gln Ala Ser Gly Tyr Arg Phe Ser Asn Phe Val Ile His
 20 25 30
 Trp Val Arg Gln Ala Pro Gly Gln Arg Phe Glu Trp Met Gly Trp Ile
 35 40 45
 Asn Pro Tyr Asn Gly Asn Lys Glu Phe Ser Ala Lys Phe Gln Asp Arg
 50 55 60
 Val Thr Phe Thr Ala Asp Thr Ser Ala Asn Thr Ala Tyr Met Glu Leu
 65 70 75 80

Arg Ser Leu Arg Ser Ala Asp Thr Ala Val Tyr Tyr Cys Ala Arg Val
85 90 95

Gly Pro Tyr Ser Trp Asp Asp Ser Pro Gln Asp Asn Tyr Tyr Met Asp
100 105 110

Val Trp Gly Lys Gly Thr Thr Val Ile Val Ser Ser
115 120

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 124 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys
1 5 10 15

Val Ser Cys Gln Ala Ser Gly Tyr Arg Phe Ser Asn Phe Val Ile His
20 25 30

Trp Val Arg Gln Ala Pro Gly Gln Arg Phe Glu Trp Met Gly Trp Ile
35 40 45

Asn Pro Tyr Asn Gly Asn Lys Glu Phe Ser Ala Lys Phe Gln Asp Arg
50 55 60

Val Thr Phe Thr Ala Asp Thr Asp Ala Asn Thr Ala Tyr Met Glu Leu
65 70 75 80

Arg Ser Leu Arg Ser Ala Asp Thr Ala Ile Tyr Tyr Cys Ala Arg Val
85 90 95

Gly Pro Tyr Thr Trp Asp Asp Ser Pro Gln Asp Asn Tyr Tyr Met Asp
100 105 110

Val Trp Gly Lys Gly Thr Lys Val Ile Val Ser Ser
115 120

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 124 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

```

Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys
1           5           10           15
Val Ser Cys Gln Ala Ser Gly Tyr Arg Phe Ser Asn Phe Val Ile His
20           25           30
Trp Val Arg Gln Ala Pro Gly Gln Arg Phe Glu Trp Met Gly Trp Ile
35           40           45
Asn Pro Tyr Asn Gly Asn Lys Glu Phe Ser Ala Lys Phe Gln Asp Arg
50           55           60
Val Thr Phe Thr Ala Asp Thr Asp Ala Asn Thr Ala Tyr Met Glu Leu
65           70           75           80
Arg Ser Leu Arg Ser Thr Asp Thr Ala Ile Tyr Tyr Cys Ala Arg Val
85           90           95
Gly Pro Tyr Thr Trp Asp Asp Ser Pro Gln Asp Asn Tyr Tyr Met Asp
100          105          110
Val Trp Gly Lys Gly Thr Lys Val Ile Val Ser Ser
115          120

```

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 130 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

```

Leu Glu Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Arg
1           5           10           15
Leu Ser Cys Val Gly Ser Gly Phe Thr Phe Ser Ser Ala Trp Met Ala
20           25           30
Trp Val Arg Gln Ala Pro Gly Arg Gly Leu Glu Trp Val Gly Leu Ile
35           40           45

```

Lys Ser Lys Ala Asp Gly Glu Thr Thr Asp Tyr Ala Thr Pro Val Lys
 50 55 60
 Gly Arg Phe Ser Ile Ser Arg Asn Asn Leu Glu Asp Thr Val Tyr Leu
 65 70 75 80
 Gln Met Asp Ser Leu Arg Ala Asp Asp Thr Ala Val Tyr Tyr Cys Ala
 85 90 95
 Thr Gln Lys Pro Arg Tyr Phe Asp Leu Leu Ser Gly Gln Tyr Arg Arg
 100 105 110
 Val Ala Gly Ala Phe Asp Val Trp Gly His Gly Thr Thr Val Thr Val
 115 120 125
 Ser Pro
 130

(2) INFORMATION FOR SEQ ID NO:70:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 130 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(11) MOLECULE TYPE: protein

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:70:

Leu Glu Glu Ser Gly Gly Gly Leu Val Lys Ala Gly Gly Ser Leu Arg
 1 5 10 15
 Leu Ser Cys Val Gly Ser Gly Phe Thr Phe Ser Ser Ala Trp Met Ala
 20 25 30
 Trp Val Gly Gln Ala Pro Gly Arg Gly Leu Glu Trp Val Gly Leu Ile
 35 40 45
 Lys Ser Lys Ala Asp Gly Glu Thr Thr Asp Tyr Ala Thr Pro Val Lys
 50 55 60
 Gly Arg Phe Ser Ile Ser Arg Asn Asn Leu Glu Asp Thr Val Tyr Leu
 65 70 75 80
 Gln Met Asp Ser Leu Arg Ala Asp Asp Thr Ala Val Tyr Tyr Cys Ala
 85 90 95
 Thr Gln Lys Pro Arg Tyr Phe Asp Leu Leu Ser Gly Gln Tyr Arg Arg
 100 105 110

Val Ala Gly Ala Phe Asp Val Trp Gly His Gly Thr Thr Val Thr Val
 115 120 125

Ser Pro
 130

(2) INFORMATION FOR SEQ ID NO:71:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 130 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

Leu Glu Glu Ser Gly Gly Gly Leu Ile Lys Pro Gly Gly Ser Leu Arg
 1 5 10 15
 Leu Ser Cys Val Gly Ser Gly Phe Thr Phe Ser Ser Ala Trp Met Thr
 20 25 30
 Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile Gly Leu Ile
 35 40 45
 Lys Ser Lys Ala Asp Gly Glu Thr Thr Asp Tyr Ala Thr Pro Val Lys
 50 55 60
 Gly Arg Phe Thr Ile Ser Arg Asn Asn Leu Glu Asn Thr Val Tyr Leu
 65 70 75 80
 Gln Met Asp Ser Leu Arg Ala Asp Asp Thr Ala Val Tyr Tyr Cys Ala
 85 90 95
 Thr Gln Lys Pro Ser Tyr Tyr Asn Leu Leu Ser Gly Gln Tyr Arg Arg
 100 105 110
 Val Ala Gly Ala Phe Asp Val Trp Gly His Gly Thr Thr Val Thr Val
 115 120 125
 Ser Pro

(2) INFORMATION FOR SEQ ID NO:72:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 125 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

```

Leu Glu Glu Ser Gly Glu Ala Val Val Gln Pro Gly Arg Ser Leu Arg
1           5           10           15
Leu Ser Cys Ala Ala Ser Gly Phe Ile Phe Arg Asn Tyr Ala Met His
20           25           30
Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Leu Ile
35           40           45
Lys Tyr Asp Gly Arg Asn Lys Tyr Tyr Ala Asp Ser Val Lys Gly Arg
50           55           60
Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met
65           70           75           80
Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp
85           90           95
Ile Gly Leu Lys Gly Glu His Tyr Asp Ile Leu Thr Ala Tyr Gly Pro
100          105          110
Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
115          120          125

```

(2) INFORMATION FOR SEQ ID NO:73:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 125 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

```

Leu Glu Gln Ser Gly Glu Ala Val Val Gln Pro Gly Thr Ser Leu Arg
1           5           10           15
Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Asn Tyr Ala Met His
20           25           30
Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Leu Ile
35           40           45

```

Lys Tyr Asp Gly Arg Asn Lys Tyr Tyr Ala Asp Ser Val Lys Gly Arg
 50 55 60
 Phe Ser Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Glu Met
 65 70 75 80
 Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp
 85 90 95
 Ile Gly Leu Lys Gly Glu His Tyr Asp Ile Leu Thr Ala Tyr Gly Pro
 100 105 110
 Asp Tyr Trp Gly Gln Gly Ala Leu Val Thr Val Ser Ser
 115 120 125

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 125 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

Leu Glu Gln Ser Gly Glu Ala Val Val Gln Pro Gly Arg Ser Leu Arg
 1 5 10 15
 Leu Ser Cys Ala Ala Ser Gly Phe Ile Phe Arg Asn Tyr Ala Met His
 20 25 30
 Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Leu Ile
 35 40 45
 Lys Tyr Asp Gly Arg Asn Lys Tyr Tyr Ala Asp Ser Val Lys Gly Arg
 50 55 60
 Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met
 65 70 75 80
 Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp
 85 90 95
 Ile Gly Leu Lys Gly Glu His Tyr Asp Ile Leu Thr Ala Tyr Gly Pro
 100 105 110
 Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 115 120 125

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 125 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

```

Leu Glu Glu Ser Gly Glu Ala Val Val Gln Pro Gly Thr Ser Leu Arg
 1             5             10             15
Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Asn Tyr Ala Met His
                20             25             30
Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Leu Ile
          35             40             45
Lys Tyr Asp Gly Arg Asn Lys Tyr Tyr Ala Asp Ser Val Lys Gly Arg
 50             55             60
Phe Ser Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Glu Met
 65             70             75             80
Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp
          85             90             95
Ile Gly Leu Lys Gly Glu His Tyr Asp Ile Leu Thr Ala Tyr Gly Pro
      100             105             110
Asp Tyr Trp Gly Gln Gly Ala Leu Val Thr Val Ser Ser
      115             120             125

```

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 125 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

```

Leu Glu Gln Ser Gly Glu Ala Val Val Gln Pro Gly Arg Ser Leu Arg
 1             5             10             15

```

Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Arg Asn Tyr Ala Met His
 20 25 30
 Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Leu Ile
 35 40 45
 Lys Tyr Asp Gly Arg Asn Lys Tyr Tyr Ala Asp Ser Val Lys Gly Arg
 50 55 60
 Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met
 65 70 75 80
 Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp
 85 90 95
 Ile Gly Leu Lys Ala Glu His Tyr Asp Ile Leu Thr Ala Tyr Gly Pro
 100 105 110
 Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 115 120 125

(2) INFORMATION FOR SEQ ID NO:77:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 125 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

Leu Glu Gln Ser Gly Glu Ala Val Val Gln Pro Gly Arg Ser Leu Arg
 1 5 10 15
 Leu Ser Cys Ala Ala Ser Gly Phe Ile Phe Arg Asn Tyr Ala Met His
 20 25 30
 Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Leu Ile
 35 40 45
 Lys Tyr Asp Gly Arg Asn Lys Tyr Tyr Ala Asp Ser Val Lys Gly Arg
 50 55 60
 Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met
 65 70 75 80
 Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asp
 85 90 95

Ile Gly Leu Lys Gly Glu His Tyr Asp Ile Leu Thr Ala Tyr Gly Pro
 100 105 110

Asp Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser
 115 120 125

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 128 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

Leu Glu Gln Ser Gly Gly Gly Val Val Lys Pro Gly Gly Ser Leu Arg
 1 5 10 15

Leu Ser Cys Glu Gly Ser Gly Phe Thr Phe Pro Asn Ala Trp Met Thr
 20 25 30

Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Val Ala Ser Ile
 35 40 45

Lys Ser Lys Phe Asp Gly Gly Ser Pro His Tyr Ala Ala Pro Val Glu
 50 55 60

Gly Arg Phe Thr Ile Ser Arg Asn Asp Leu Glu Asp Lys Val Phe Leu
 65 70 75 80

Gln Met Asn Gly Leu Lys Ala Glu Asp Thr Gly Val Tyr Tyr Cys Ala
 85 90 95

Thr Arg Tyr Pro Arg Tyr Ser Glu Met Met Gly Gly Val Arg Lys His
 100 105 110

Phe Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Ser Val Ser Ser
 115 120 125

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 128 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

Leu Glu Glu Ser Gly Gly Gly Val Val Lys Pro Gly Gly Ser Leu Arg
 1 5 10 15
 Leu Ser Cys Glu Gly Ser Gly Phe Thr Phe Pro Asn Ala Trp Met Thr
 20 25 30
 Trp Val Arg Gln Ser Pro Gly Lys Gly Leu Glu Trp Val Ala Ser Ile
 35 40 45
 Lys Ser Lys Phe Asp Gly Gly Ser Pro His Tyr Ala Ala Pro Val Glu
 50 55 60
 Gly Arg Phe Thr Ile Ser Arg Asn Asp Leu Glu Asp Lys Val Phe Leu
 65 70 75 80
 Gln Met Asn Gly Leu Lys Ala Glu Asp Thr Gly Val Tyr Tyr Cys Ala
 85 90 95
 Thr Arg Tyr Pro Arg Tyr Ser Glu Met Met Gly Gly Val Arg Lys His
 100 105 110
 Phe Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Ser Val Ser Ser
 115 120 125

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 122 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

Leu Glu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Arg Ser Leu Arg
 1 5 10 15
 Val Ser Cys Glu Ala Ser Gly Phe Thr Phe Ser Ser Tyr Glu Met Asn
 20 25 30
 Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Gln Ile
 35 40 45
 Ser Ser Ser Gly Ser Arg Thr Tyr Tyr Ala Asp Ser Val Lys Gly Arg

50 55 60
 Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr Leu Glu Met
 65 70 75 80
 Thr Ser Leu Arg Val Asp Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gly
 85 90 95
 Arg Arg Leu Val Thr Phe Gly Gly Val Val Ser Gly Gly Asn Ile Trp
 100 105 110
 Gly Gln Gly Thr Met Val Thr Val Ser Ser
 115 120

(2) INFORMATION FOR SEQ ID NO:81:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 126 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

Leu Glu Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg Ser Leu Arg
 1 5 10 15
 Leu Ser Cys Ala Gly Ser Gly Phe Asn Phe Ser Asp Asp Thr Met His
 20 25 30
 Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Val Ile
 35 40 45
 Ser Tyr Glu Gly Ser Asp Lys Tyr Tyr Ala Asp Ser Val Lys Gly Arg
 50 55 60
 Phe Thr Ile Ser Arg Asp Asn Ser Glu Asn Thr Leu Tyr Leu Gln Met
 65 70 75 80
 Asp Ser Leu Arg Ala Asp Asp Thr Ala Leu Tyr Tyr Cys Ala Arg Asn
 85 90 95
 Thr Arg Glu Asn Ile Glu Ala Asp Gly Thr Ala Tyr Tyr Ser Tyr Tyr
 100 105 110
 Met Asp Val Trp Gly Lys Gly Thr Thr Val Thr Val Ser Ser
 115 120 125

(2) INFORMATION FOR SEQ ID NO:82:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 107 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
 1 5 10 15
 Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Asn Tyr Leu Ala
 20 25 30
 Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro Arg Leu Leu Ile Tyr Ala
 35 40 45
 Ala Ser Thr Leu Gln Pro Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
 50 55 60
 Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp
 65 70 75 80
 Val Ala Thr Tyr Tyr Cys Gln Lys Tyr Asn Ser Ala Pro Arg Thr Phe
 85 90 95
 Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr
 100 105

(2) INFORMATION FOR SEQ ID NO:83:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 106 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Ile Gly Asp Arg
 1 5 10 15
 Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Asn Asn Tyr Leu Ala
 20 25 30
 Trp Tyr Gln Gln Arg Pro Gly Lys Val Pro Arg Leu Leu Ile Tyr Ala

35 40 45
 Ala Ser Thr Leu Gln Ser Gly Val Pro Thr Arg Phe Ser Gly Ser Gly
 50 55 60
 Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp
 65 70 75 80
 Val Ala Thr Tyr Tyr Cys Gln Lys Tyr Asn Ser Val Pro Arg Thr Phe
 85 90 95
 Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
 100 105

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 107 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
 1 5 10 15
 Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Ser Asn Tyr Leu Ala
 20 25 30
 Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Leu Ile Tyr Ala
 35 40 45
 Ala Ser Thr Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
 50 55 60
 Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp
 65 70 75 80
 Val Ala Thr Tyr Tyr Cys Gln Lys Tyr Asn Ser Ala Pro Arg Thr Phe
 85 90 95
 Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr
 100 105

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 106 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:85:

Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Ile Gly Asp Arg
 1 5 10 15
 Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Ile Asn Asn Tyr Leu Ala
 20 25 30
 Trp Tyr Gln Gln Arg Pro Gly Lys Ala Pro Asn Leu Leu Ile Tyr Ala
 35 40 45
 Ala Ser Thr Leu Gln Ser Gly Val Pro Pro Arg Phe Ser Gly Ser Gly
 50 55 60
 Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp
 65 70 75 80
 Val Ala Thr Tyr Tyr Cys Gln Lys Tyr Asn Ser Val Pro His Thr Phe
 85 90 95
 Gly Gly Gly Thr Lys Val Glu Ile Lys Arg
 100 105

(2) INFORMATION FOR SEQ ID NO:86:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 108 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:86:

Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg
 1 5 10 15
 Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ile Ser Asn Tyr Leu
 20 25 30
 Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr
 35 40 45

Gly Val Ser Asn Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser Gly Ser
 50 55 60

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu
 65 70 75 80

Asp Phe Ala Val Tyr Ser Cys Gln Gln Tyr Gly Thr Ser Pro Trp Thr
 85 90 95

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr
 100 105

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 107 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg
 1 5 10 15

Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Asn Asn Tyr Leu
 20 25 30

Ala Trp Tyr Gln Gln Arg Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr
 35 40 45

Gly Ala Ser Asn Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser Gly Ser
 50 55 60

Gly Ser Gly Thr Ala Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu
 65 70 75 80

Asp Val Ala Ile Tyr Tyr Cys Gln Gln Tyr His Ser Ser Pro Tyr Thr
 85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
 100 105

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 108 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

```

Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg
1           5           10           15
Ala Thr Leu Ser Cys Arg Ala Ser His Arg Val Asn Asn Asn Phe Leu
20           25           30
Ala Trp Tyr Gln Gln Lys Pro Gln Ala Pro Arg Leu Leu Ile Ser Gly
35           40           45
Ala Ser Thr Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly
50           55           60
Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Asp Asp
65           70           75           80
Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Asp Ser Pro Leu Tyr Ser
85           90           95
Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
100          105

```

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 105 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

```

Glu Leu Thr Gln Ser Pro Ala Ser Val Ser Ala Ser Val Gly Asp Thr
1           5           10           15
Val Thr Ile Thr Cys Arg Ala Ser Gln Asp Ile His Asn Trp Leu Ala
20           25           30
Trp Tyr Gln Gln Gln Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala
35           40           45
Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Arg Gly
50           55           60

```

Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp
 65 70 75 80
 Phe Ala Thr Tyr Tyr Cys Gln Gln Gly Asn Ser Phe Pro Lys Phe Gly
 85 90 95
 Pro Gly Thr Val Val Asp Ile Lys Arg
 100 105

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 107 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg
 1 5 10 15
 Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Leu Ser Asn Asn Tyr Leu
 20 25 30
 Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr
 35 40 45
 Gly Ser Ser Thr Arg Gly Thr Gly Ile Pro Asp Arg Phe Ser Gly Gly
 50 55 60
 Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu
 65 70 75 80
 Asp Phe Ala Val Tyr Tyr Cys Gln His Tyr Gly Asn Ser Val Tyr Thr
 85 90 95
 Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
 100 105

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 104 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

Gln Ser Pro Asp Thr Leu Ser Leu Asn Pro Gly Glu Arg Ala Thr Leu
 1 5 10 15
 Ser Cys Arg Ala Ser His Arg Ile Ser Ser Lys Arg Leu Ala Trp Tyr
 20 25 30
 Gln His Lys Arg Gly Gln Ala Pro Arg Leu Leu Ile Tyr Val Cys Pro
 35 40 45
 Asn Arg Ala Gly Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly
 50 55 60
 Thr Asp Phe Thr Leu Thr Tyr Ser Arg Leu Glu Pro Glu Asp Phe Ala
 65 70 75 80
 Met Tyr Tyr Cys Gln Tyr Tyr Gly Gly Ser Ser Tyr Thr Phe Gly Gln
 85 90 95
 Gly Thr Lys Val Glu Ile Thr Arg
 100

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 104 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

Gln Ser Pro Ser His Leu Ser Leu Ser Pro Gly Glu Arg Ala Ile Leu
 1 5 10 15
 Ser Cys Arg Ala Ser Gln Arg Val Ser Ala Pro Tyr Leu Ala Trp Tyr
 20 25 30
 Gln Gln Arg Pro Gly Gln Ala Pro Arg Leu Val Ile Tyr Gly Ala Ser
 35 40 45
 Thr Arg Ala Thr Asp Ile Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly
 50 55 60
 Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu Asp Phe Ala
 65 70 75 80

Ile Tyr Tyr Cys Gln Val Tyr Gly Gln Ser Pro Val Leu Phe Gly Gln
 85 90 95

Gly Thr Lys Leu Glu Met Lys Arg
 100

(2) INFORMATION FOR SEQ ID NO:93:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 105 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Asp Arg Ala Thr Leu
 1 5 10 15

Ser Cys Arg Ala Ser Gln Ser Leu Ser Ser Ser Phe Leu Ala Trp Tyr
 20 25 30

Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Ser Ala Ser
 35 40 45

Met Arg Ala Thr Gly Ile Pro Asp Arg Phe Arg Gly Ser Val Ser Gly
 50 55 60

Thr Asp Phe Thr Leu Thr Ile Thr Arg Leu Glu Pro Glu Asp Phe Ala
 65 70 75 80

Val Tyr Tyr Cys Gln Arg Phe Gly Thr Ser Pro Leu Tyr Thr Phe Gly
 85 90 95

Gln Gly Thr Lys Leu Glu Met Lys Arg
 100 105

(2) INFORMATION FOR SEQ ID NO:94:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 104 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu
 1 5 10 15
 Ser Cys Arg Ala Ser Gln Ser Phe Ser Ser Asn Phe Leu Ala Trp Tyr
 20 25 30
 Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Val His Pro
 35 40 45
 Asn Arg Ala Thr Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly
 50 55 60
 Thr Asp Phe Thr Leu Thr Ile Arg Arg Leu Glu Pro Glu Asp Phe Ala
 65 70 75 80
 Val Tyr Tyr Cys Gln Gln Tyr Gly Ala Ser Leu Val Ser Phe Gly Pro
 85 90 95
 Gly Thr Lys Val His Ile Lys Arg
 100

(2) INFORMATION FOR SEQ ID NO:95:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 108 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg
 1 5 10 15
 Ala Thr Phe Ser Cys Arg Ser Ser His Ser Ile Arg Ser Arg Arg Val
 20 25 30
 Ala Trp Tyr Gln His Lys Pro Gly Gln Ala Pro Arg Leu Val Ile His
 35 40 45
 Gly Val Ser Asn Arg Ala Ser Gly Ile Ser Asp Arg Phe Ser Gly Ser
 50 55 60
 Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Arg Val Glu Pro Glu
 65 70 75 80
 Asp Phe Ala Leu Tyr Tyr Cys Gln Val Tyr Gly Ala Ser Ser Tyr Thr
 85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Arg Lys Arg Thr
 100 105

(2) INFORMATION FOR SEQ ID NO:96:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 108 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Thr Pro Gly Glu Arg
 1 5 10 15
 Ala Thr Leu Ser Cys Arg Thr Ser His Ser Ile Arg Ser Arg Arg Leu
 20 25 30
 Ala Trp Tyr Gln Val Lys Gly Gly Gln Ala Pro Arg Leu Leu Ile Tyr
 35 40 45
 Gly Val Ser Asn Arg Ala Gly Gly Ile Pro Asp Arg Phe Ser Gly Ser
 50 55 60
 Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu
 65 70 75 80
 Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Arg Tyr Thr
 85 90 95
 Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
 100 105

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 107 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

Glu Leu Thr Gln Ala Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg
 1 5 10 15

Ala Thr Phe Ser Cys Arg Ser Ser His Ser Ile Arg Ser Arg Arg Val
 20 25 30
 Arg Trp Tyr Gln His Lys Pro Gly Gln Ala Pro Arg Leu Val Ile His
 35 40 45
 Gly Val Ser Asn Arg Ala Ser Gly Ile Ser Asp Arg Phe Ser Gly Ser
 50 55 60
 Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Arg Val Glu Pro Glu
 65 70 75 80
 Asp Phe Ala Leu Tyr Tyr Cys Gln Val Tyr Gly Ala Ser Ser Tyr Thr
 85 90 95
 Phe Gly Gln Gly Thr Lys Leu Glu Arg Lys Arg
 100 105

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 108 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

Glu Leu Thr Gln Ala Pro Gly Thr Leu Ser Leu Ser Pro Gly Asp Arg
 1 5 10 15
 Ala Thr Phe Ser Cys Arg Ser Ser His Asn Ile Arg Ser Arg Arg Val
 20 25 30
 Ala Trp Tyr Gln His Lys Pro Gly Gln Ala Pro Arg Leu Val Ile His
 35 40 45
 Gly Val Ser Asn Arg Ala Ser Gly Ile Ser Asp Arg Phe Ser Gly Ser
 50 55 60
 Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Arg Leu Glu Pro Glu
 65 70 75 80
 Asp Phe Ala Leu Tyr Tyr Cys Gln Val Tyr Gly Ala Ser Ser Tyr Thr
 85 90 95
 Phe Gly Gln Gly Thr Lys Leu Asp Phe Lys Arg Thr
 100 105

(1) SEQUENCE CHARACTERISTICS:

- (ii) MOLECULE TYPE: protein

Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg
1 5 10 15

Ala Thr Leu Ser Cys Arg Ala Gly Gln Ser Ile Ser Ser Asn Tyr Leu
20 25 30

Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr
35 40 45

Gly Ala Ser Asn Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Asp Phe Thr Leu Ser Ile Ser Arg Leu Glu Pro Glu
65 70 75 80

Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Thr Ser Pro Tyr Thr
85 90 95

Phe Gly Gln Gly Thr Gln Leu Asp Ile Lys Arg Thr
100 105

(i) SEQUENCE CHARACTERISTICS:

- (ii) MOLECULE TYPE: protein

Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Leu
1 5 10 15

Ser Cys Arg Ala Ser Gln Ser Leu Ser Asn Asn Tyr Leu Ala Trp Tyr
20 25 30

Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu Ile Tyr Gly Ser Ser
 35 40 45
 Thr Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser Gly Gly Gly Ser Gly
 50 55 60
 Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu Asp Phe Ala
 65 70 75 80
 Val Tyr Tyr Cys Gln Gln Tyr Gly Asn Ser Val Tyr Thr Phe Gly Gln
 85 90 95
 Gly Thr Lys Leu Glu Ile Lys Arg
 100

(2) INFORMATION FOR SEQ ID NO:101:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 106 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
 1 5 10 15
 Val Thr Ile Thr Cys Arg Thr Ser Gln Gly Ile Ser Asn Tyr Leu Ala
 20 25 30
 Trp Tyr Gln Gln Lys Pro Gly Lys Val Pro Lys Leu Leu Ile Tyr Gly
 35 40 45
 Ala Ser Thr Leu Gln Ser Gly Gly Pro Ser Arg Phe Ser Gly Ser Gly
 50 55 60
 Ser Gly Thr Asp Phe Thr Leu Thr Ile Asn Ser Leu Gln Pro Glu Asp
 65 70 75 80
 Val Ala Thr Tyr Ser Cys Gln Asn Tyr Asp Ser Ala Pro Trp Thr Phe
 85 90 95
 Gly Gln Gly Thr Lys Val Asp Ile Lys Arg
 100 105

(2) INFORMATION FOR SEQ ID NO:102:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 108 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
 1 5 10 15
 Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Asn Tyr Leu Asn
 20 25 30
 Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala
 35 40 45
 Ala Ser Ser Leu Gln Arg Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
 50 55 60
 Ser Gly Thr Asp Phe Thr Leu Ser Ile Ser Ser Leu Gln Pro Glu Asp
 65 70 75 80
 Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Ile Pro Pro Leu Thr
 85 90 95
 Phe Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr
 100 105

(2) INFORMATION FOR SEQ ID NO:103:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 107 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
 1 5 10 15
 Val Thr Ile Thr Cys Arg Ala Ser Gln Asn Ile Asn Asn Tyr Leu Asn
 20 25 30
 Trp Tyr Gln Gln Lys Pro Gly Glu Ala Pro Lys Leu Leu Ile His Thr
 35 40 45

Ala Phe Asn Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Thr Ala
 50 55 60

Ser Gly Thr Glu Phe Thr Leu Thr Ile Arg Ser Leu Gln Pro Glu Asp
 65 70 75 80

Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Pro Tyr Thr Phe
 85 90 95

Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr
 100 105

(2) INFORMATION FOR SEQ ID NO:104:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 107 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
 1 5 10 15

Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr Leu Asn
 20 25 30

Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala
 35 40 45

Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
 50 55 60

Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp
 65 70 75 80

Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Pro Tyr Thr Phe
 85 90 95

Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
 100 105

(2) INFORMATION FOR SEQ ID NO:105:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 107 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

```

Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
1           5           10           15
Val Thr Ile Thr Cys Arg Ala Ser Gln Ser Ile Ser Ser Tyr Leu Asn
          20           25           30
Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala
          35           40           45
Ala Ser Ser Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
          50           55           60
Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp
          65           70           75           80
Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Pro Gln Thr Phe
          85           90           95
Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
          100          105

```

(2) INFORMATION FOR SEQ ID NO:106:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 104 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

```

Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile
1           5           10           15
Thr Cys Arg Ala Ser Gln Thr Ile Ser Ser Tyr Leu Asn Trp Tyr Gln
          20           25           30
Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Ser
          35           40           45
Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Gly Gly Ser Gly Thr
          50           55           60

```

Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr
65 70 75 80

Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Pro Tyr Thr Phe Gly Gln Gly
85 90 95

Thr Lys Leu Glu Ile Lys Arg Thr
100

(2) INFORMATION FOR SEQ ID NO:107:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 107 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
1 5 10 15

Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Arg Asn Tyr Leu Asn
20 25 30

Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Asp
35 40 45

Ala Ser Asn Ser Glu Thr Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
50 55 60

Ser Gly Arg Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp
65 70 75 80

Val Ala Thr Tyr Tyr Cys Gln Gln His Gln Asn Val Pro Leu Thr Phe
85 90 95

Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr
100 105

(2) INFORMATION FOR SEQ ID NO:108:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 107 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
 1 5 10 15
 Val Thr Ile Thr Cys Gln Ala Ser Gln Asp Ile Ser Asn His Leu Asn
 20 25 30
 Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Asp
 35 40 45
 Ala Ser Asn Leu Glu Thr Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
 50 55 60
 Ser Gly Thr Asp Phe Thr Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp
 65 70 75 80
 Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Asp Asn Leu Pro Leu Thr Phe
 85 90 95
 Gly Gly Gly Thr Lys Val Glu Ile Lys Arg Thr
 100 105

(2) INFORMATION FOR SEQ ID NO:109:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 108 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg
 1 5 10 15
 Ile Thr Ile Thr Cys Arg Ala Ser Gln Thr Ile Asn Asn Tyr Leu Asn
 20 25 30
 Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Tyr Gly
 35 40 45
 Ala Ser Asn Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly Ser Gly
 50 55 60
 Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp
 65 70 75 80

Phe Ala Thr Tyr Phe Cys Gln Gln Ser Tyr Asn Thr Pro Pro Trp Thr
 85 90 95

Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr
 100 105

(2) INFORMATION FOR SEQ ID NO:110:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 108 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg
 1 5 10 15

Ala Thr Leu Ser Cys Arg Ala Ser Gln Arg Val Asn Ser Asn Tyr Leu
 20 25 30

Ala Trp Tyr Gln Gln Lys Pro Gly Gln Thr Pro Arg Val Val Ile Tyr
 35 40 45

Ser Thr Ser Arg Arg Ala Thr Gly Val Pro Asp Arg Phe Ser Gly Ser
 50 55 60

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu
 65 70 75 80

Asp Phe Ala Val Tyr Tyr Cys Gln Gln Phe Gly Asp Ala Gln Tyr Thr
 85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr
 100 105

(2) INFORMATION FOR SEQ ID NO:111:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 93 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Arg Val Asn Ser Asn
 1 5 10 15
 Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Thr Pro Arg Val Val
 20 25 30
 Ile Tyr Ser Thr Ser Arg Arg Ala Thr Gly Val Pro Asp Arg Phe Ser
 35 40 45
 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
 50 55 60
 Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Phe Gly Asp Ala Gln
 65 70 75 80
 Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
 85 90

(2) INFORMATION FOR SEQ ID NO:112:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 104 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:

Thr Gln Ser Pro Ser Ser Val Ser Ala Ser Val Gly Asp Thr Val Thr
 1 5 10 15
 Phe Thr Cys Arg Ala Ser Gln Asp Ile Arg Asn Tyr Leu Asn Trp Tyr
 20 25 30
 His Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile Ser Asp Ala Ser
 35 40 45
 Asp Leu Glu Ile Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Ala
 50 55 60
 Thr Tyr Phe Ser Phe Thr Ile Ser Ser Leu Gln Pro Glu Asp Ile Gly
 65 70 75 80
 Thr Tyr Tyr Cys Gln Gln Tyr Ala Asp Leu Ile Thr Phe Gly Gly Gly
 85 90 95
 Thr Lys Val Glu Ile Lys Arg Thr
 100

(2) INFORMATION FOR SEQ ID NO:113:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 96 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:

```

Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val
1           5           10           15
Gly Thr Asn Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg
20           25           30
Leu Leu Ile Phe Asp Ala Ser Thr Arg Asp Thr Tyr Ile Pro Asp Thr
35           40           45
Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ala Leu Thr Ile Ser Ser
50           55           60
Leu Gln Ser Glu Asp Phe Gly Phe Tyr Tyr Cys Gln Gln Tyr Asp Asn
65           70           75           80
Trp Pro Pro Thr Phe Gly Gln Gly Thr Lys Leu Glu Val Lys Arg Thr
85           90           95

```

(2) INFORMATION FOR SEQ ID NO:114:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 107 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:

```

Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Asp Arg
1           5           10           15
Ala Thr Phe Ser Cys Arg Ser Ser His Asn Ile Arg Ser Arg Arg Val
20           25           30
Ala Trp Tyr Gln His Lys Pro Gly Gln Ala Pro Arg Leu Val Ile His

```

35 40 45
 Gly Val Ser Asn Arg Ala Ser Gly Ile Ser Asp Arg Phe Ser Gly Ser
 50 55 60
 Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Arg Leu Glu Pro Glu
 65 70 75 80
 Asp Phe Ala Leu Tyr Tyr Cys Gln Val Tyr Gly Ala Ser Ser Tyr Thr
 85 90 95
 Phe Gly Gln Gly Thr Lys Leu Asp Phe Lys Arg
 100 105

(2) INFORMATION FOR SEQ ID NO:115:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 107 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:115:

Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg
 1 5 10 15
 Ala Thr Phe Ser Cys Arg Ser Ser His Asn Ile Arg Ser Arg Arg Val
 20 25 30
 Ala Trp Tyr Gln His Lys Pro Gly Gln Ala Pro Arg Leu Val Ile His
 35 40 45
 Gly Val Ser Asn Arg Ala Thr Gly Ile Ser Asp Arg Phe Ser Gly Ser
 50 55 60
 Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Arg Leu Glu Pro Glu
 65 70 75 80
 Asp Phe Ala Leu Tyr Tyr Cys Gln Val Tyr Gly Ala Ser Ser Tyr Thr
 85 90 95
 Phe Gly Gln Gly Thr Lys Leu Asp Phe Lys Arg
 100 105

(2) INFORMATION FOR SEQ ID NO:116:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 107 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:

```

Glu Leu Thr Gln Ser Pro Asp Thr Leu Ser Leu Asn Val Gly Glu Arg
1           5           10           15
Ala Thr Leu Ser Cys Arg Ala Ser His Arg Ile Ser Ser Arg Arg Leu
20           25           30
Ala Trp Tyr Gln His Lys Arg Gly Gln Ala Pro Arg Leu Leu Ile Tyr
35           40           45
Gly Val Ser Ser Arg Ala Gly Gly Val Pro Asp Arg Phe Ser Gly Ser
50           55           60
Gly Ser Gly Thr Asp Phe Ser Leu Thr Ile Ser Arg Leu Glu Pro Glu
65           70           75           80
Asp Phe Ala Met Tyr Tyr Cys Gln Thr Tyr Gly Gly Ser Ser Tyr Thr
85           90           95
Phe Gly Gln Gly Thr Lys Val Asp Ile Lys Arg
100          105

```

(2) INFORMATION FOR SEQ ID NO:117:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:117:

```

Glu Leu Thr Gln Ser Pro Asp Thr Leu Ser Leu Asn Ala Gly Glu Arg
1           5           10           15
Ala Thr Leu Ser Cys Arg Ala Ser His Arg Ile Ser Ser Arg Arg Leu
20           25           30
Ala Trp Tyr Gln His Lys Arg Gly Gln Ala Pro Arg Leu Leu Ile Tyr
35           40           45

```

Gly Val Ser Asn Arg Ala Gly Gly Val Pro Asp Arg Phe Ser Gly Ser
 50 55 60

Gly Ser Gly Thr Asp Phe Ser Leu Thr Ile Ser Arg Leu Glu Pro Glu
 65 70 75 80

Asp Phe Ala Ile Tyr Tyr Cys Gln Thr Tyr Gly Gly Ser Ser Tyr Thr
 85 90 95

Phe Gly Gln Gly Thr Thr Val Asp Ile Lys Arg
 100 105

(2) INFORMATION FOR SEQ ID NO:118:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 107 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:118:

Glu Leu Thr Gln Ser Pro Asp Thr Leu Ser Leu Asn Thr Gly Glu Arg
 1 5 10 15

Ala Thr Leu Ser Cys Arg Ala Ser His Arg Ile Gly Ser Arg Arg Leu
 20 25 30

Ala Trp Tyr Gln His Arg Arg Gly Gln Ala Pro Arg Leu Leu Ile Tyr
 35 40 45

Gly Val Ser Asn Arg Ala Gly Gly Val Pro Asp Arg Phe Ser Gly Ser
 50 55 60

Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu
 65 70 75 80

Asp Phe Ala Ile Tyr Tyr Cys Gln Thr Tyr Gly Gly Ser Ser Tyr Thr
 85 90 95

Phe Gly Gln Gly Thr Lys Val Asp Ile Lys Arg
 100 105

(2) INFORMATION FOR SEQ ID NO:119:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 107 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:119:

```

Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Thr Pro Gly Glu Arg
1           5           10           15
Ala Ile Leu Ser Cys Lys Thr Ser His Asn Ile Trp Ser Arg Arg Leu
20           25           30
Ala Trp Tyr Gln Leu Lys Ser Gly Gln Ala Pro Arg Leu Leu Ile Tyr
35           40           45
Gly Val Ser Lys Arg Ala Gly Gly Ile Pro Asp Arg Phe Ser Gly Ser
50           55           60
Gly Ser Ala Thr Asp Phe Thr Leu Thr Ile Ser Arg Val Glu Pro Glu
65           70           75           80
Asp Phe Ala Val Tyr Tyr Cys Gln Thr Tyr Gly Gly Ser Ala Tyr Thr
85           90           95
Phe Gly Gln Gly Thr Lys Leu Asp Ile Lys Arg
100          105

```

(2) INFORMATION FOR SEQ ID NO:120:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 107 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:

```

Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Thr Pro Gly Glu Arg
1           5           10           15
Ala Ile Leu Ser Cys Lys Thr Ser His Asn Ile Trp Ser Arg Arg Leu
20           25           30
Ala Trp Tyr Gln Leu Lys Ser Gly Gln Ala Pro Arg Leu Leu Ile Tyr
35           40           45
Gly Val Ser Lys Arg Ala Gly Gly Ile Pro Asp Arg Phe Ser Gly Ser
50           55           60

```


Gly Ser Ala Thr Asp Phe Thr Leu Thr Ile Ser Arg Val Glu Pro Glu
65 70 75 80

Asp Phe Ala Val Tyr Tyr Cys Gln Thr Tyr Gly Gly Ser Ala Tyr Thr
85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105

(2) INFORMATION FOR SEQ ID NO:121:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 107 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:

Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Ser Thr Pro Gly Glu Arg
1 5 10 15

Ala Ile Leu Ser Cys Lys Thr Ser His Asn Ile Trp Ser Arg Arg Leu
20 25 30

Ala Trp Tyr Gln Val Lys Ser Gly Leu Pro Pro Arg Leu Leu Ile His
35 40 45

Gly Val Ser Arg Arg Ala Gly Gly Ile Pro Asp Arg Phe Ser Gly Ser
50 55 60

Gly Ser Ala Arg Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Ala
65 70 75 80

Asp Phe Ala Val Tyr Tyr Cys Gln Thr Tyr Gly Gly Ser Ser Tyr Ser
85 90 95

Phe Gly Gln Gly Thr Lys Leu Asp Phe Asn Arg
100 105

(2) INFORMATION FOR SEQ ID NO:122:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 107 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:122:

Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Asn Pro Gly Glu Arg
 1 5 10 15
 Ala Val Leu Ser Cys Arg Thr Ser Arg Asn Ile Trp Ser Arg Arg Leu
 20 25 30
 Ala Trp Tyr Gln Val Arg Arg Gly Gln Ala Pro Arg Leu Leu Ile His
 35 40 45
 Gly Val Ser Lys Arg Ala Gly Gly Val Pro Asp Arg Phe Ser Gly Ser
 50 55 60
 Gly Ser Ala Arg Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu
 65 70 75 80
 Asp Phe Ala Val Tyr Phe Cys Gln Thr Tyr Gly Gly Ser Ser Tyr Thr
 85 90 95
 Phe Gly Gln Gly Asn Lys Leu Asp Ile Arg Arg
 100 105

(2) INFORMATION FOR SEQ ID NO:123:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 126 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:123:

Gln Val Lys Leu Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
 1 5 10 15
 Ala Ser Val Lys Val Ser Cys Gln Ala Ser Gly Tyr Arg Phe Ser Asn
 20 25 30
 Phe Val Leu His Trp Ala Arg Gln Ala Pro Gly His Arg Pro Glu Trp
 35 40 45
 Met Gly Trp Ile Asn Pro Ala Asn Gly Val Thr Glu Ile Pro Pro Lys
 50 55 60
 Phe Gln Asp Arg Val Ser Leu Thr Arg Asp Thr Ser Ala Gly Thr Val
 65 70 75 80

Tyr Leu Glu Leu Thr Asn Leu Arg Phe Ala Asp Thr Ala Val Tyr Tyr
85 90 95

Cys Ala Arg Val Gly Glu Trp Thr Trp Asp Asp Ser Pro Gln Asp Asn
100 105 110

Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Thr Val
115 120 125

(2) INFORMATION FOR SEQ ID NO:124:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 125 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:124:

Gln Val Lys Leu Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
1 5 10 15

Ala Ser Val Lys Val Ser Cys Gln Ala Ser Gly Tyr Arg Phe Ser Asn
20 25 30

Phe Val Leu His Trp Ala Arg Gln Ala Pro Gly His Arg Pro Glu Trp
35 40 45

Met Gly Trp Ile Asn Pro Ala Asn Gly Val Thr Glu Ile Ser Pro Lys
50 55 60

Phe Gln Asp Arg Val Ser Leu Thr Gly Asp Thr Ser Ala Ser Thr Val
65 70 75 80

Tyr Leu Glu Leu Arg Asn Leu Arg Phe Ala Asp Thr Ala Val Tyr Tyr
85 90 95

Cys Ala Arg Val Gly Glu Trp Thr Trp Asp Asp Ser Pro Gln Asp Asn
100 105 110

Tyr Tyr Met Asp Val Trp Gly Arg Gly Thr Thr Val Thr
115 120 125

(2) INFORMATION FOR SEQ ID NO:125:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 124 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:125:

```

Gln Val Lys Leu Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
1           5           10           15
Ala Ser Val Lys Val Ser Cys Gln Ala Ser Gly Tyr Arg Phe Ser Asn
20           25           30
Phe Val Leu His Trp Ala Arg Gln Ala Pro Gly His Arg Pro Glu Trp
35           40           45
Met Gly Trp Ile Asn Pro Ala Asn Gly Val Thr Glu Ile Ser Pro Lys
50           55           60
Phe Gln Asp Arg Val Ser Leu Thr Gly Asp Thr Ser Ala Ser Thr Val
65           70           75           80
Tyr Leu Glu Leu Arg Ser Leu Arg Phe Ala Asp Thr Ala Val Tyr Tyr
85           90           95
Cys Ala Arg Val Gly Glu Trp Thr Trp Asp Asp Ser Pro Gln Asp Asn
100          105          110
Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val
115          120

```

(2) INFORMATION FOR SEQ ID NO:126:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 124 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:126:

```

Gln Val Lys Leu Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
1           5           10           15
Ala Ser Val Lys Ile Ser Cys Gln Ala Ser Gly Tyr Arg Phe Thr Asn
20           25           30
Phe Val Leu His Trp Ala Arg Gln Ala Pro Gly Gln Arg Pro Glu Trp
35           40           45

```

Met Gly Trp Phe Asn Pro Ala Asn Gly Ile Lys Glu Ile Ser Pro Lys
 50 55 60

Phe Gln Asp Arg Val Ser Phe Thr Gly Asp Thr Ser Ala Ser Thr Ala
 65 70 75 80

Tyr Val Glu Leu Arg Asn Leu Arg Ser Ala Asp Thr Ala Val Tyr Tyr
 85 90 95

Cys Ala Arg Val Gly Pro Trp Thr Trp Asp Asp Ser Pro Gln Asp Asn
 100 105 110

Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val
 115 120

(2) INFORMATION FOR SEQ ID NO:127:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 124 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:127:

Gln Val Lys Leu Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
 1 5 10 15

Ala Ser Val Lys Val Ser Cys Gln Ala Ser Gly Tyr Arg Phe Ser Asn
 20 25 30

Phe Val Leu His Trp Ala Arg Gln Ala Pro Gly His Arg Pro Glu Trp
 35 40 45

Met Gly Trp Ile Asn Pro Ala Asn Gly Val Thr Glu Ile Ser Pro Lys
 50 55 60

Phe Gln Asp Arg Val Ser Leu Thr Gly Asp Thr Ser Ala Ser Thr Val
 65 70 75 80

Tyr Leu Glu Leu Arg Asn Leu Arg Phe Ala Asp Thr Ala Val Tyr Tyr
 85 90 95

Cys Ala Arg Val Gly Glu Trp Thr Trp Asp Asp Phe Pro Gln Asp Asn
 100 105 110

Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val
 115 120

(2) INFORMATION FOR SEQ ID NO:128:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 125 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:128:

Gln Val Lys Leu Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
 1 5 10 15

Ala Ser Val Lys Leu Ser Cys Gln Ala Ser Gly Tyr Arg Phe Ser Asn
 20 25 30

Phe Val Leu His Trp Ala Arg Gln Ala Pro Gly His Arg Pro Glu Trp
 35 40 45

Met Gly Trp Ile Asn Pro Ala Asn Gly Val Thr Glu Ile Ser Pro Lys
 50 55 60

Phe Gln Asp Arg Val Ser Leu Thr Gly Asp Thr Ser Ala Ser Thr Val
 65 70 75 80

Tyr Leu Glu Leu Arg Asn Leu Arg Phe Ala Asp Thr Ala Val Tyr Tyr
 85 90 95

Cys Ala Arg Val Gly Glu Trp Thr Trp Asp Asp Ser Pro Gln Asp Asn
 100 105 110

Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Thr
 115 120 125

(2) INFORMATION FOR SEQ ID NO:129:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 125 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:129:

Gln Val Lys Leu Leu Glu Gln Ser Gly Thr Glu Val Lys Lys Pro Gly
 1 5 10 15

Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Arg Phe Thr Asn
 20 25 30
 Phe Pro Leu His Trp Val Arg Gln Ala Pro Gly Gln Arg Pro Glu Trp
 35 40 45
 Met Gly Trp Ile Lys Ile Val Asn Gly Glu Lys Lys Tyr Ser Gln Lys
 50 55 60
 Phe Val Asp Arg Val Thr Phe Thr Gly Asp Thr Ser Ala Asn Thr Ala
 65 70 75 80
 Tyr Met Glu Val Arg Gly Leu Arg Ser Ala Asp Thr Ala Thr Tyr Tyr
 85 90 95
 Cys Ala Arg Val Gly Glu Trp Thr Trp Asp Met Asp Pro Gln Ala Asn
 100 105 110
 Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Thr
 115 120 125

(2) INFORMATION FOR SEQ ID NO:130:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 124 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:130:

Gln Val Lys Leu Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
 1 5 10 15
 Ala Ser Val Lys Val Ser Cys Gln Ala Ser Gly Tyr Arg Phe Ser Asn
 20 25 30
 Phe Val Ile His Trp Val Arg Gln Ala Pro Gly Gln Arg Phe Glu Trp
 35 40 45
 Met Gly Trp Ile Asn Pro Tyr Asn Gly Asn Lys Glu Phe Ser Ala Lys
 50 55 60
 Phe Arg Asp Arg Val Thr Phe Thr Ala Asp Thr Asp Ala Asn Thr Ala
 65 70 75 80
 Tyr Met Glu Leu Arg Ser Leu Arg Ser Ala Asp Thr Ala Ile Tyr Tyr
 85 90 95

Cys Ala Arg Val Gly Pro Tyr Thr Trp Asp Asp Ser Pro Gln Asp Asn
 100 105 110

Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val
 115 120

(2) INFORMATION FOR SEQ ID NO:131:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 124 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:131:

Gln Val Lys Leu Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
 1 5 10 15

Ala Ser Val Lys Val Ser Cys Gln Ala Ser Gly Tyr Arg Phe Ser Asn
 20 25 30

Phe Val Leu His Trp Ala Arg Gln Ala Pro Thr Gln Asp Leu Glu Trp
 35 40 45

Met Gly Trp Ile Asn Pro Ala Asn Gly Val Lys Glu Ile Ser Pro Lys
 50 55 60

Phe Gln Asp Arg Val Ser Leu Thr Gly Asp Thr Ser Ala Ser Thr Val
 65 70 75 80

Tyr Leu Glu Leu Arg Ser Leu Arg Phe Ala Asp Thr Ala Val Tyr Tyr
 85 90 95

Cys Ala Arg Val Gly Glu Trp Thr Trp Asp Asp Ser Pro Gln Asp Asn
 100 105 110

Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val
 115 120

(2) INFORMATION FOR SEQ ID NO:132:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 124 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:132:

Gln Val Lys Leu Leu Glu Gln Ser Gly Ala Glu Val Lys Lys Pro Gly
 1 5 10 15
 Ala Ser Val Lys Val Ser Cys Gln Ala Ser Gly Tyr Arg Phe Ser Asn
 20 25 30
 Phe Val Leu His Trp Ala Arg Gln Ala Pro Gly His Arg Pro Glu Trp
 35 40 45
 Met Gly Trp Ile Asn Pro Ala Asn Gly Val Thr Glu Ile Pro Pro Lys
 50 55 60
 Phe Gln Asp Arg Val Ser Leu Thr Arg Asp Thr Ser Ala Gly Thr Val
 65 70 75 80
 Tyr Leu Glu Leu Thr Asn Leu Arg Phe Ala Asp Thr Ala Val Tyr Tyr
 85 90 95
 Cys Ala Arg Val Gly Glu Trp Thr Trp Asp Asp Ser Pro Gln Asp Asn
 100 105 110
 Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val
 115 120

(2) INFORMATION FOR SEQ ID NO:133:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:133:

TCGAGGGTCG GTCGGTCTCT AGACGGTCGG TCGGTCA

37

(2) INFORMATION FOR SEQ ID NO:134:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 base pairs
- (B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:134:

CTAGTGACCG ACCGACCGTC TAGAGACCGA CCGACCC

37

(2) INFORMATION FOR SEQ ID NO:135:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:135:

CGGTCGGTCG GTCCTCGAGG GTCGGTCGGT CT

32

(2) INFORMATION FOR SEQ ID NO:136:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 40 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:136:

CTAGAGACCG ACCGACCCTC GAGGACCGAC CGACCGAGCT

40

(2) INFORMATION FOR SEQ ID NO:137:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:137:

CAAGGAGACA GGATCCATGA AATAC

25

(2) INFORMATION FOR SEQ ID NO:138:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:138:

AGGGCGAATT GGATCCCGGG CCCCC

25

(2) INFORMATION FOR SEQ ID NO:139:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 29 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:139:

CTAGTCATCA TCATCATCAT TAAGCTAGC

29

(2) INFORMATION FOR SEQ ID NO:140:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 29 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:140:

CTAGGCTAGC TTAATGATGA TGATGATGA

29

(2) INFORMATION FOR SEQ ID NO:141:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: /label- J

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 13
- (D) OTHER INFORMATION: /label- ZC

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:141:

Ser Ile Ser Ile Gly Pro Gly Arg Ala Phe Tyr Thr Gly
 1 5 10

(2) INFORMATION FOR SEQ ID NO:142:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 126 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:142:

Leu Leu Glu Ser Gly Pro Gly Leu Val Lys Pro Ser Glu Thr Leu Ser
 1 5 10 15
 Leu Thr Cys Thr Val Ser Gly Gly Ser Leu Ser Ser Phe Asp Trp Asn
 20 25 30
 Trp Ile Arg Gln Pro Ala Gly Lys Gly Leu Glu Trp Ile Gly Arg Ile
 35 40 45
 Tyr Pro Ser Gly Asn Thr His Tyr Asn Pro Ser Leu Arg Ser Arg Val
 50 55 60
 Thr Met Ser Arg Asp Thr Ser Lys Asn Gln Phe Ser Val Lys Leu Thr
 65 70 75 80
 Ser Val Thr Ala Ala Asp Thr Ala Leu Tyr Tyr Cys Ala Arg Glu Asn
 85 90 95
 Thr Gly Arg Thr Ile Glu Glu Ile Gly Asn Phe Phe Asp Ile Trp Gly
 100 105 110
 Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
 115 120 125

(2) INFORMATION FOR SEQ ID NO:143:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 122 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:143:

Leu Leu Lys Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Arg
 1 5 10 15
 Leu Ser Cys Val Ile Ser Ala Phe Ser Phe Ser Gly Tyr Asn Ile Asn
 20 25 30
 Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ser Ile
 35 40 45
 Ser Met Ser Thr Gly Ser Leu Ser Tyr Ala Asp Ser Met Lys Gly Arg
 50 55 60
 Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Val Tyr Leu Glu Met
 65 70 75 80
 Ser Ser Leu Thr Ala Glu Asp Thr Ala Met Tyr Tyr Cys Ala Ala Arg
 85 90 95
 Thr Pro Leu Val Gly Arg Ala Leu Asp Ile Trp Gly Gln Gly Thr Val
 100 105 110
 Val Thr Val Ser Ser Ala Ser Thr Lys Gly
 115 120

(2) INFORMATION FOR SEQ ID NO:144:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 132 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:144:

Leu Leu Glu Ser Gly Gly Gly Leu Val Lys Pro Gly Gly Ser Leu Arg
 1 5 10 15
 Leu Ser Cys Ser Ala Ser Gly Phe Thr Phe Ser Ser Tyr Gly Met Asn
 20 25 30
 Trp Val Arg Gln Ala Pro Gly Lys Gly Pro Glu Trp Val Ala Tyr Ile
 35 40 45
 Ser Ser Ser Arg Lys Tyr Thr Glu Tyr Ala Asp Ser Val Lys Gly Arg
 50 55 60
 Phe Thr Ile Ser Arg Glu Asn Ala Lys Tyr Ser Val Phe Leu Gln Leu

65 70 75 80
 Asp Ser Leu Thr Ala Glu Asp Thr Ala Ile Tyr Tyr Cys Ala Arg Gly
 85 90 95
 Arg Asp Phe Tyr Ser Gly Phe Gly Arg Arg Asp Asp Phe His Leu His
 100 105 110
 Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val Thr Val Ser Ser Ala
 115 120 125
 Ser Thr Lys Gly
 130

(2) INFORMATION FOR SEQ ID NO:145:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 126 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:145:

Leu Leu Glu Gln Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu
 1 5 10 15
 Arg Ile Ser Cys Val Ala Ser Gly Asp Ile Phe Tyr Ser Tyr Ala Met
 20 25 30
 Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala Ser
 35 40 45
 Ile Ser Gly Thr Gly Gly Ser Asn Tyr Tyr Ala Asp Ser Val Lys Gly
 50 55 60
 Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Ser Thr Leu Tyr Leu Gln
 65 70 75 80
 Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu Tyr Tyr Cys Ala Arg
 85 90 95
 Asp Arg Gly Pro Arg Ile Gly Ile Arg Gly Trp Phe Asp Ser Trp Gly
 100 105 110
 Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly
 115 120 125

(2) INFORMATION FOR SEQ ID NO:146:

- (1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 124 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(11) MOLECULE TYPE: protein

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:146:

Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg
1 5 10 15
Leu Ser Cys Ala Ala Ser Gly Phe Leu Tyr Ser Ser Phe Ala Met Ser
20 25 30
Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Ala Trp Val Ser Thr Ile
35 40 45
Ser Ala Ser Gly Gly Ser Thr Lys Tyr Ala Asp Ser Val Lys Gly Arg
50 55 60
Phe Ile Ile Ser Arg Asp Asn Ser Lys Asn Thr Ile Tyr Leu Gln Met
65 70 75 80
Asp Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Lys Asn
85 90 95
Phe Arg Ala Phe Ala Arg Asp Pro Trp Gly Asp Trp Gly Gln Gly Thr
100 105 110
Leu Val Thr Val Ser Ser Ala Ser Ala Ser Thr Lys
115 120

(2) INFORMATION FOR SEQ ID NO:147:

- (1) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(11) MOLECULE TYPE: protein

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:147:

Met Ala Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
1 5 10 15
Glu Arg Val Ile Val Ser Cys Arg Ala Ser Gln Ser Val Ser Ser Asn

20 25 30
 Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
 35 40 45
 Ile Tyr Gly Ala Ser Asn Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
 65 70 75 80
 Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Gly
 85 90 95
 Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr
 100 105

(2) INFORMATION FOR SEQ ID NO:148:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 112 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:148:

Met Ala Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15
 Glu Arg Ala Thr Phe Ser Cys Arg Ser Ser His Ser Ile His Thr Arg
 20 25 30
 Arg Val Ala Trp Tyr Gln His Lys Pro Gly Gln Ala Pro Arg Leu Val
 35 40 45
 Ile His Gly Val Ser Asn Arg Ala Ser Gly Ile Ser Asp Arg Phe Ser
 50 55 60
 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Thr Arg Val Glu
 65 70 75 80
 Pro Glu Asp Phe Ala Leu Tyr Tyr Cys Gln Val Tyr Gly Ala Ser Ser
 85 90 95
 Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Arg Lys Arg Thr Val Val
 100 105 110

(2) INFORMATION FOR SEQ ID NO:149:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 111 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:149:

```

Met Ala Glu Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
 1           5           10           15
Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Asn Gly
          20           25           30
Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
          35           40           45
Ile Tyr Gly Ala Ser Thr Arg Ala Thr Asp Ile Pro Asp Arg Phe Ser
          50           55           60
Gly Ser Gly Ser Gly Ala Asp Phe Thr Leu Ala Ile Ser Arg Leu Glu
          65           70           75           80
Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Ala Gly Ser His
          85           90           95
Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala
          100          105          110

```

(2) INFORMATION FOR SEQ ID NO:150:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 111 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:150:

```

Met Ala Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1           5           10           15
Asp Arg Val Thr Ile Thr Cys Arg Pro Ser Gln Gly Ile Gly Arg Phe
          20           25           30

```

Phe Asn Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Asn Leu Leu Ile
 35 40 45
 Tyr Ala Ala Asp Ile Leu Gln Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
 65 70 75 80
 Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Tyr Ser Thr Pro Tyr
 85 90 95
 Thr Phe Gly Gln Gly Thr Arg Leu Asp Ile Lys Arg Thr Val Ala
 100 105 110

(2) INFORMATION FOR SEQ ID NO:151:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 112 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:151:

Met Ala Glu Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15
 Asp Arg Val Thr Ile Thr Cys Arg Ala Ser Gln Gly Val Ser Ser Ser
 20 25 30
 Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Val
 35 40 45
 Ile Phe Gly Ala Tyr Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
 50 55 60
 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg Leu Glu
 65 70 75 80
 Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Pro
 85 90 95
 Ile Thr Phe Gly Pro Gly Thr Lys Val Asp Ile Lys Arg Thr Val Ala
 100 105 110

(2) INFORMATION FOR SEQ ID NO:152:

- (1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 729 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 9..715

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:152:

AGCTTACC ATG GGT GTG CCC ACT CAG GTC CTG GGG TTG CTG CTG CTG TGG	50
Met Gly Val Pro Thr Gln Val Leu Gly Leu Leu Leu Trp	
1 5 10	
CTT ACA GAT GCC AGA TGT GAG ATC GTT CTC ACG CAG TCT CCA GGC ACC	98
Leu Thr Asp Ala Arg Cys Glu Ile Val Leu Thr Gln Ser Pro Gly Thr	
15 20 25 30	
CTG TCT CTG TCT CCA GGG GAA AGA GCC ACC TTC TCC TGT AGG TCC AGT	146
Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Phe Ser Cys Arg Ser Ser	
35 40 45	
CAC AGC ATT CGC AGC CGC CGC GTA GCC TGG TAC CAG CAC AAA CCT GGC	194
His Ser Ile Arg Ser Arg Arg Val Ala Trp Tyr Gln His Lys Pro Gly	
50 55 60	
CAG GCT CCA AGG CTG GTC ATA CAT GGT GTT TCC AAT AGG GCC TCT GGC	242
Gln Ala Pro Arg Leu Val Ile His Gly Val Ser Asn Arg Ala Ser Gly	
65 70 75	
ATC TCA GAC AGG TTC AGC GGC AGT GGG TCT GGG ACA GAC TTC ACT CTC	290
Ile Ser Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu	
80 85 90	
ACC ATC ACC AGA GTG GAG CCT GAA GAC TTT GCA CTG TAC TAC TGT CAG	338
Thr Ile Thr Arg Val Glu Pro Glu Asp Phe Ala Leu Tyr Tyr Cys Gln	
95 100 105 110	
GTC TAT GGT GCC TCC TCG TAC ACT TTT GGC CAG GGG ACC AAA CTG GAG	386
Val Tyr Gly Ala Ser Ser Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu	
115 120 125	
AGG AAA CGA ACT GTG CCT GCA CCA TCT GTC TTC ATC TTC CCG CCA TCT	434
Arg Lys Arg Thr Val Pro Ala Pro Ser Val Phe Ile Phe Pro Pro Ser	
130 135 140	

GAT GAG CAG TTG AAA TCT GGG ACT GCC TCT GTT GTG TGC CTG CTG AAT Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn 145 150 155	482
AAC TTC TAT CCC AGA GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala 160 165 170	530
CTC CAA TCG GGT AAC TCC CAG GAG AGT GTC ACA GAG CAG GAC AGC AAG Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys 175 180 185 190	578
GAC AGC ACC TAC AGC CTC AGC AGC ACC CTG ACG CTG AGC AAA GCA GAC Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp 195 200 205	626
TAC GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC ACC CAT CAG GGC CTG Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu 210 215 220	674
AGT TCG CCC GTC ACA AAG AGC TTC AAC AGG GGA GAG TGT TA ATTCTAGAGA Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys 225 230 235	725
ATTG	729

(2) INFORMATION FOR SEQ ID NO:153:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 235 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:153:

Met Gly Val Pro Thr Gln Val Leu Gly Leu Leu Leu Trp Leu Thr 1 5 10 15
Asp Ala Arg Cys Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser 20 25 30
Leu Ser Pro Gly Glu Arg Ala Thr Phe Ser Cys Arg Ser Ser His Ser 35 40 45
Ile Arg Ser Arg Arg Val Ala Trp Tyr Gln His Lys Pro Gly Gln Ala 50 55 60
Pro Arg Leu Val Ile His Gly Val Ser Asn Arg Ala Ser Gly Ile Ser 65 70 75 80

Asp Arg Phe Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
 85 90 95
 Thr Arg Val Glu Pro Glu Asp Phe Ala Leu Tyr Tyr Cys Gln Val Tyr
 100 105 110
 Gly Ala Ser Ser Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Arg Lys
 115 120 125
 Arg Thr Val Pro Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
 130 135 140
 Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
 145 150 155 160
 Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
 165 170 175
 Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
 180 185 190
 Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
 195 200 205
 Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
 210 215 220
 Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 225 230 235

(2) INFORMATION FOR SEQ ID NO:154:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 3282 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 15..452

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:154:

AATTCGCCGC CACC ATG GAA TGG AGC TGG GTC TTT CTC TTC TTC CTG TCA
 Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu Ser
 1 5 10

1/56

SHINE-DALGARNO MET

GGCCGCAAATTCTATTTCAAGGAGACAGTCATAATG
CGTTTAAGATAAAGTTCCTCTGTCAGTATTAC

LEADER SEQUENCE

AAATACCTATTGCCTACGGCAGCCGCT
TTTATGGATAACGGATGCCGTCGGCGA

LEADER SEQUENCE

GGATTGTTATTACTCGCTGCCCAACCAG
CCTAACATAATGAGCGACGGGTTGGTC

LINKER

LINKER

NCOI	V _H BACKBONE	XHOI	SPEI
------	-------------------------	------	------

CCATGGCCCAGGTGAACTGCTCGAGATTTCTAGACTAGT
GGTACCGGGTCCACTTTGACGAGCTCTAAAGATCTGATCA

TyrProTyrAspValProAspTyrAlaSer STOP LINKER
TACCCGTACGACGTTCCGGACTACGGTTCCTTAATAGAATTCTG
ATGGGCATGCTGCAAGGCCTGATGCCAAGAATTATCTTAAGCAGCT

FIG.1

2/56

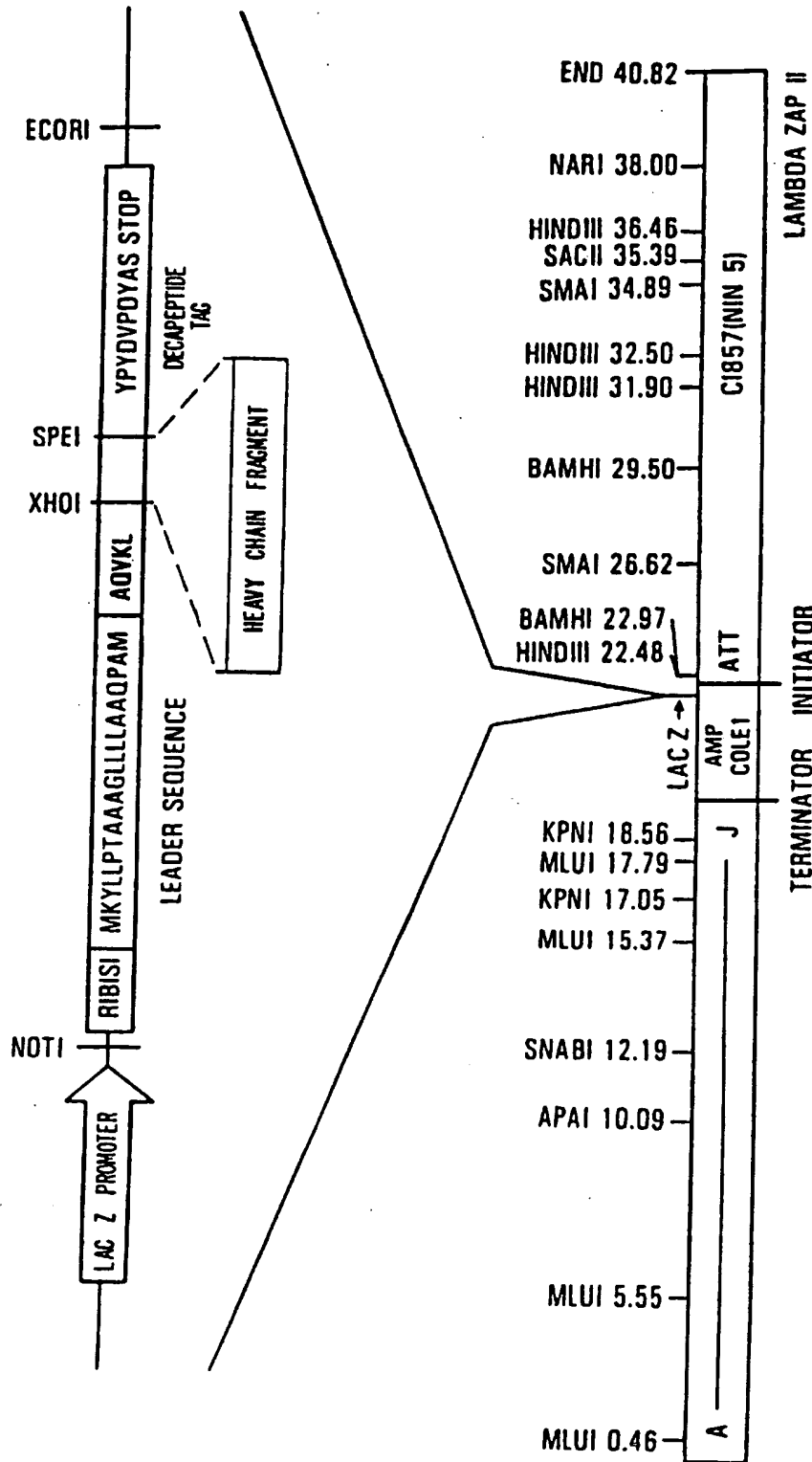


FIG.2

3/56

ECOR I SHINE-DALGARNO MET

TGAATTC TAACTAGTCGCCAAGGAGACAGTCAATAATGAAAT
TCGAACTTAAGATTGTATCAGCGGTTCCCTCTGTCTGTCAGTATTACTTTA

LEADER SEQUENCE

ACCTATTGCCCTACGGCAGCCGCTGGATTGTTATTACTCGCTGCCCAACCAG
TGGATAACGGATGCCGTCGGCGACCTAACCAATAATGAGCGACGGTTGGTC

NCO I SAC I XBA I Not I
CCATGGCCGAGCTCGTCAGTCTAGAGTTAAGCGGCCG
GGTACCGGCTCGAGCAGTCAAGATCTCAATTGCGCGGCGAGCT

FIG.3

4/56

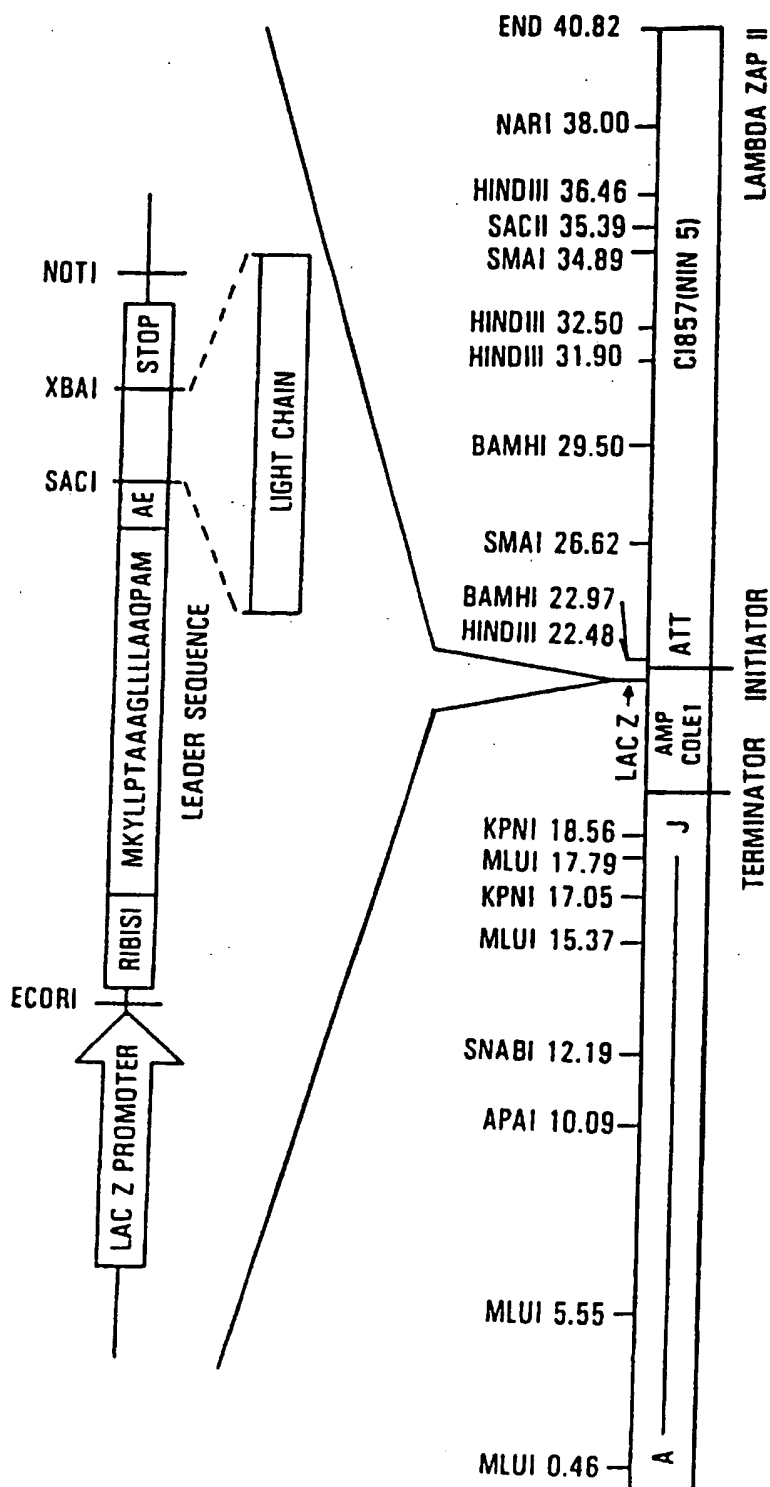


FIG.4

SUBSTITUTE SHEET (RULE 26)

5/56

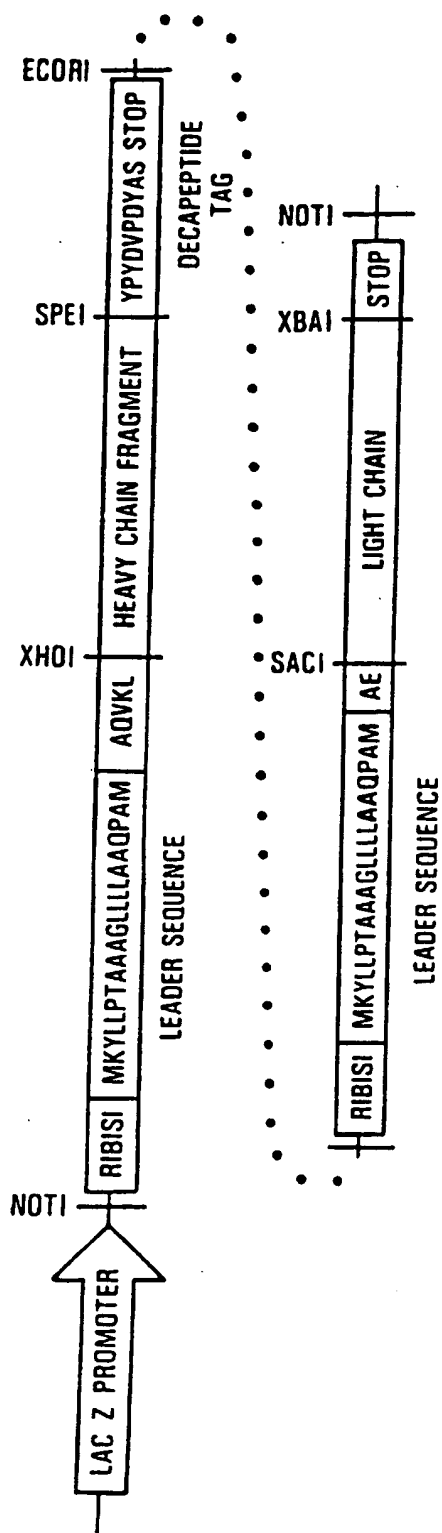


FIG. 5

6/56

CLONE NO.	FAB CONCT ($\mu\text{g/ml}$)	ELISA TITER	<u>p24 ASSAY</u>		<u>SYNCYTIA ASSAY</u>
			<u>MN</u>	<u>IIIB</u>	<u>MN</u>
1	1.8	1:8	-	-	-
2	3.1	1:64	-	-	-
3	4.1	1:32	-	-	-
4	25.0	1:16	40	80	>128
5	2.4	1:128	-	-	-
6	4.0	1:64	-	-	-
7	4.5	1:64	20	20	32
8	14.0	1:256	20	20	-
11	11.0	1:128	-	-	-
12	6.0	1:64	80	40	>128
13	6.1	1:128	80	80	-
18	0.9	1:128	-	20	-
20	6.9	1:256	-	-	32
21	8.5	1:32	20	20	32
22	8.6	1:64	20	20	-
24	0.7	1:32	-	-	-
27	10.0	1:64	20	20	32
29	16.0	1:1024	-	-	-
31	9.3	1:128	-	-	-
35	8.9	1:64	-	-	-
2F5mAb	10.0		40	160	
2F5Fab	5.0		40	20	
F58mAb	10.0		160	40	
F58F(ab') ₂	200.0		40	20	

FIG. 6

7/56

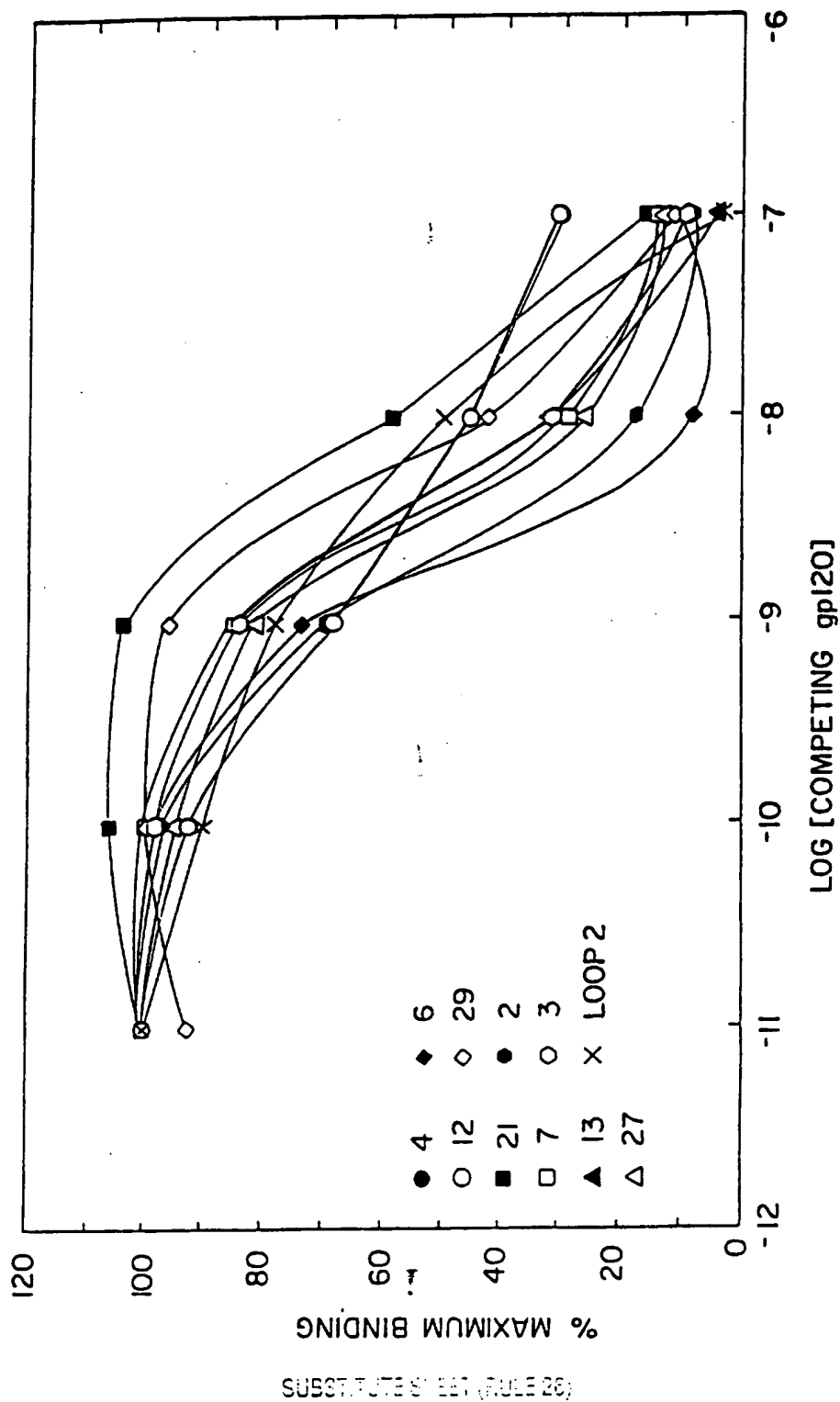


FIG.7

8/56

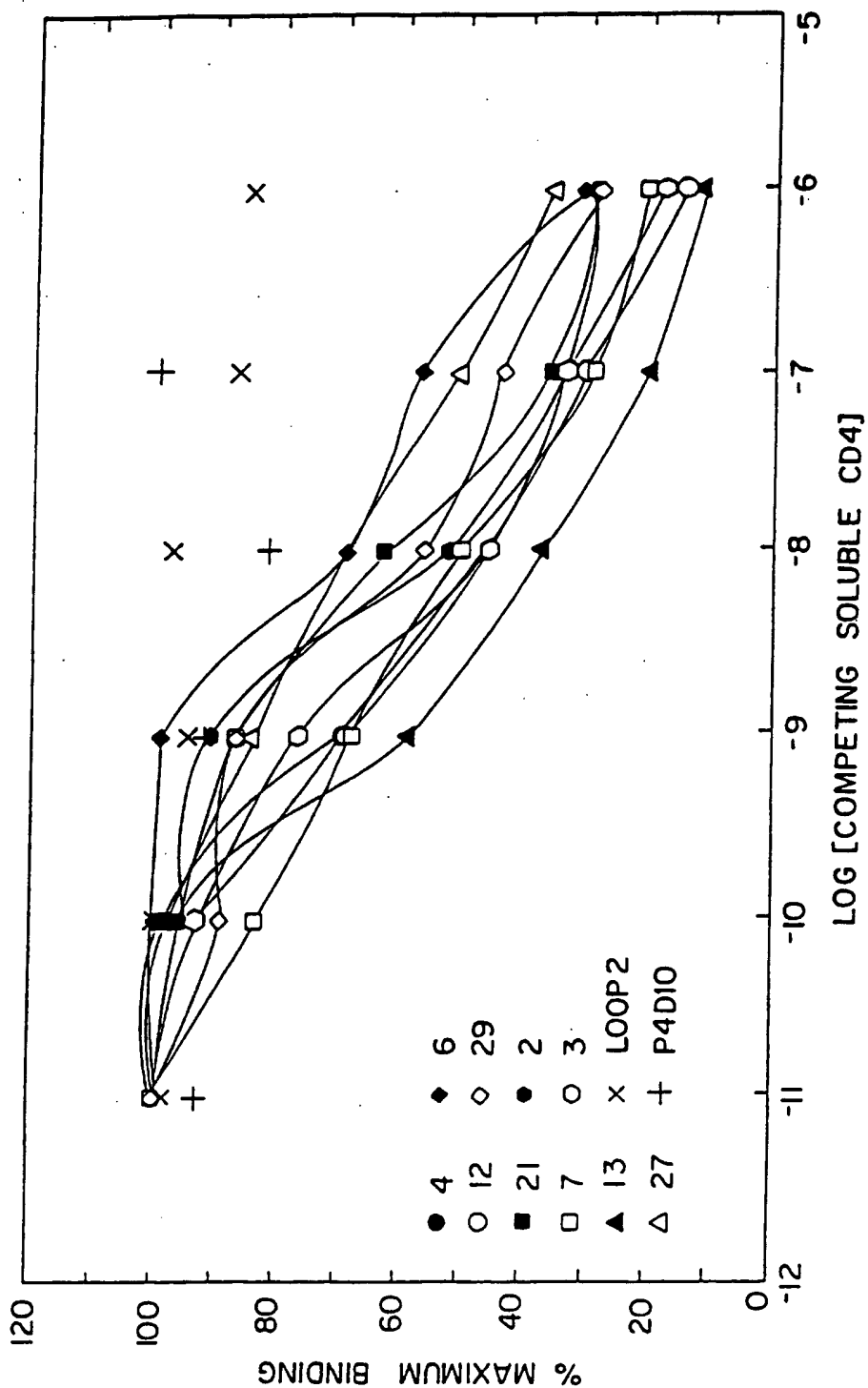
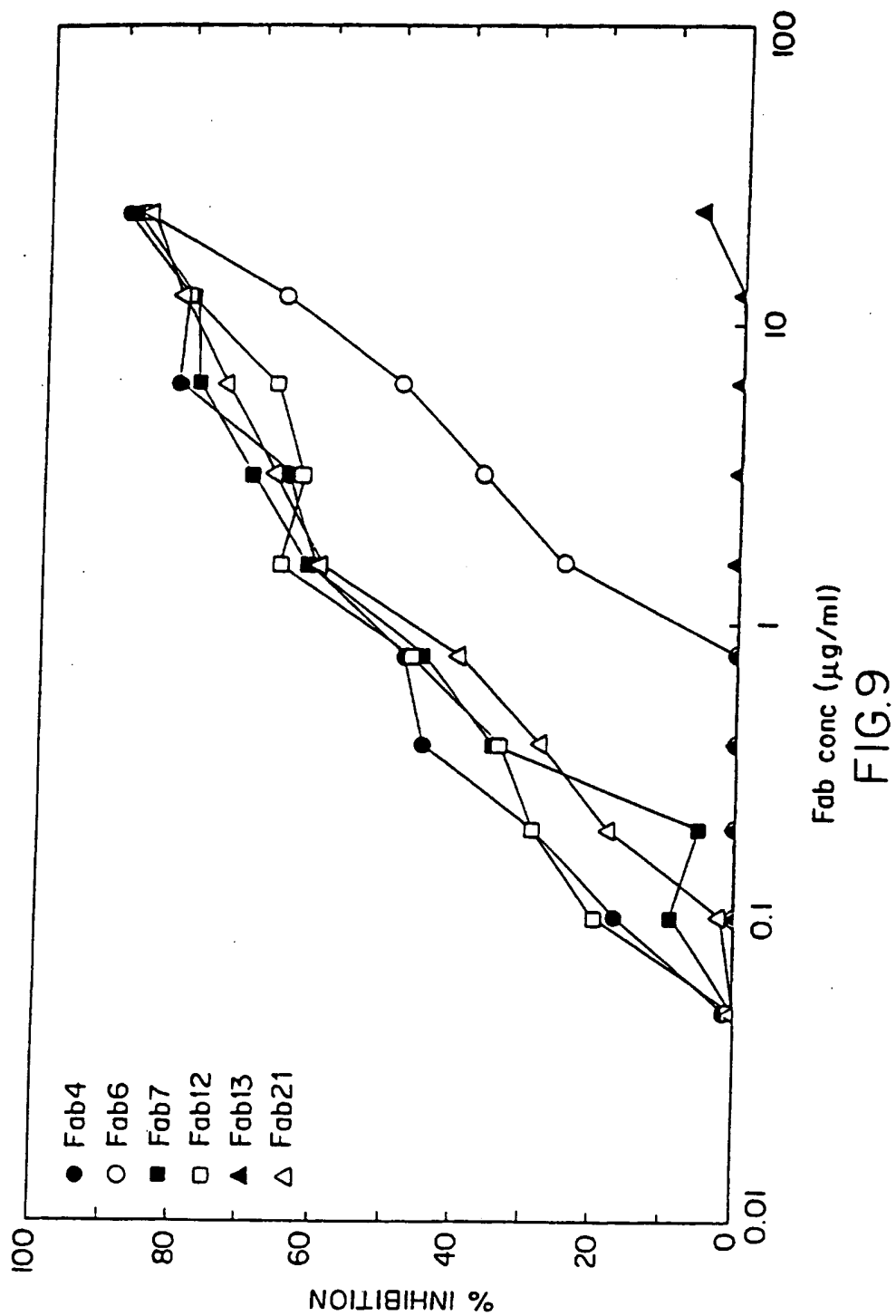


FIG.8

(SUBSTITUTE SHEET (RULE 26))

9/56



SUBSTITUTE SHEET (RULE 60)

10/5.6

C clone	FR1	CDR1	FR2	CDR2
b1	LEESGTEFKPPGSSVKVSKASGGTGG	DYASNYALS	WVRQAPGGGLEYIG	GITPTSGSADYAKQFQG
b2	...AAVQK...R...Q...D NF...	...V...	...WM...	...T...S...
b14	...A.V.K...I.S.F...
b24	...AAVQK...R...Q...D NF...	...V...	...WM...	...T...S...
B2	...Q...A.V.K...T...S...	...H...	...M...	...T...S...
B30	LEESGGRLVKPGGSLRLSCEGSGTFT	NAWMT	WVRQSPGKGLEWVA	SIKSKFDGGSPPHYAAPVEG
b3	...Q...G...
b5	...G...
B20	...A...	S...
S2	...G...	S...
S3	...G...	S...
S5	...G...	S...
S7	...G...	S...
b4	LEQSGAEVVKPGASVKVSCQASGYRFS	NFVIH	WVRQAPGGRFEWVG	WINPYNGNKEFSAKFQD
b7
b12
b21
b6	LEESGGGLVKPGGSLRLSCVSGSFTFS	SAWMA	WVRQAPGRGLEWVG	LIKSKADGETTDYATPVKG
b20	...A...G...	...
s6	...I...K...I...	...
b8	LEESGEAVVQPGGRSLRLSCAASGFI	FR NYAMH	WVRQAPGKGLEWVA	LIKYDGRNKYYADSVKG
b13	...Q...
b18	...Q...
b22	...T...
b27	...T...
B26	...Q...
B8	...Q...
B35	...Q...
S4
b11	LEQSGGGVVKPGGSLRLSCEGSGTFT	NAWMT	WVRQSPGKGLEWVA	SIKSKFDGGSPPHYAAPVEG
b29	...E...
s8	LEESGGGLVQPGGRSLRVSCASGFTFS	SYEMN	WVRQAPGKGLEWVS	QISSSGSRTYYADSVKG
100p35	LEQSGGGVVKPGGRSLRLSCAGSGFNFS	DDTMH	WVRQAPGKGLEWVA	VISYEGSDKYYADSVKG

FIG.10A

11/56

FR3	CDR3	FR4	J gene	SEQ ID NO
RVTISADRFPTILYNELSLRIEDTAIYYCAR	ERRRGMNPRALRGALDF	WGQGTRVVFVSP	JH3	53
...A.P.L...I...DD...V...	...V...V...	...I...		54
...A.A...R.V...S...V.F...	...V...V...	...I...		55
...A.P.L...I...DD...V...	...V...V...	...I...		56
...H.E...V...S.DQH...I...	...I...	...I...		57
...H.E...V...S.DQH...I...	...I...	...I...		58
RFSISRNDLEDKMFLEMSGLKAEDTGVYYCAT	KYPRYSMDVTGVRNHFYMDV	WGKGTTTVIVSS	JH6	59
...T...L...L...F...MA...	...F...MA...	...I...		60
...T...L...L...MA...L...	...MA...L...	...		61
...T...L...L...MA...L...	...MA...L...	...		62
...T...L...L...MA...L...	...MA...L...	...		63
...T...Y...I...L...Y...MR...	...Y...MR...	...		64
...T...Y...I...L...Y...MR...	...Y...MR...	...		65
RVFTTADTSANTAYMELSLRSADTAIYYCAR	VGYPYSDDDSPQDNYYMDV	WGKGTTTVIVSS	JH6	66
...D...D...I...I...I...	...I...I...	...K...		67
...D...D...I...I...I...	...I...I...	...K...		68
RFSISRNNLEDTVYLQMDSLRADDTAVYYCAT	QKPRYFDLLSGQYRRVAGAFDV	WGHGTTTVTVSP	JH3	69
...T...N...N...S...YN...	...S...YN...	...		70
...T...N...N...S...YN...	...S...YN...	...		71
RFTISRDNKNTLYLQMNSLRAEDTAIYYCAR	DIGLKGHYDILTAYGPDY	WGQGLTVTVSS	JH4	72
...S...E...E...A...	...A...	...A...		73
...S...E...E...A...	...A...	...A...		74
...S...E...E...A...	...A...	...A...		75
...S...E...E...A...	...A...	...A...		76
...S...E...E...A...	...A...	...A...		77
...S...E...E...A...	...A...	...A...		78
RFTISRNDLEDKVFLQMNGLKAEDTGVYYCAT	RYPRYSEMGGVRKHFYMDV	WGKGTTTVSVSS	JH6	79
...S...E...E...A...	...A...	...A...		80
RFTISRDNKNSLYLEMTSLRVDDTAIYYCAR	GRRLVTFGGVVSGGNI	WGQGTMTVTVSS	JH3	81
RFTISRDNSENNTLYLQMDSLRADDTALYYCAR	NTRENIEADGTAYYSYYMDV	WGKGTTTVTVSS	JH6	82

FIG. 10B

12/56

Clone	FR1	CDR1	FR2	CDR2
b1	ELTQSPSSLSASVGDRTITC	RASQGISNYLA	WYQQKPGKVPRLIIY	AASTLQP
b2I.....N....R.....S
b14I.....N....K.....S
B2I.....N....R.....S
b24GT..L.P.E.A.LS.SVISNYLAA.N....	GV.NRAT
B30GT..L.P.E.A.LS.SVSNLYLAQA.....	G..NRAT
b3	ELTQSPGTLSPGERATLSC	RASHRVNNFLA	WYQQKPGQAPRLIIS	GASTRAT
b5	ELTQSPASVSASVGDITVTTC	RASQDIHNWLA	WYQQKPGKAPKLLIY	AASSLQS
B20	ELTQSPGTLSPGERATLSC	RASQSLSNLYLA	WYQQKPGQAPRLIIY	GSSTRGT
S2	QSPDTLSLNPGERATLSC	RASHRISSKRLA	WYQHKRGQAPRLIIY	VCPNRAG
S3	QSPSHLSLSPGERATLSC	RASQSVSAPYLA	WYQGRPGQAPRLVIY	GASTRAT
S5	QSPGTLSPGDRATLSC	RASQSLSSSFLA	WYQQKPGQAPRLIIY	SASMRAT
S7	QSPGTLSPGERATLSC	RASQSFSSNFLA	WYQQKPGQAPRLIIY	VHPNRAT
b4	ELTQSPGTLSPGERATFSC	RSSHISIRRRVA	WYQHKPGQAPRLVIH	GVSNRAS
b7I.....L..L....V.G.....G
b12A.....R....L.Y
b21A.....D.....N.....
b6	ELTQSPGTLSPGERATLSC	RAGQSISSNYLA	WYQQKPGQAPRLIIY	GASNRAT
S6A.....A.i.i.S..L.N....KV.K....S.T..
b20SS..A.V.D.V.i.i.TS.G..NYLATLQS
b8	ELTQSPSSLSASVGDRTITC	RASQSISSNYLN	WYQQKPGKAPKLLIY	AASSLQR
b18N.N....E.....H	T.FN..S
b22S....S
b27S....S
B35S....S
S4T..S....S
b13Q....D.R....	D..NSET
B26Q....D..H..	D..N.ET
B8T.N....	G..N..S
b11	ELTQSPGTLSPGERATLSC	RASQSVNSNYLA	WYQQKPGQTPRVIIY	STSRAT
b29
S8	TQSPSSVSASVGDITVTFTC	RASQDIRNYLN	WYHOKPGKAPKLLIS	DASDLEI
loop35	SPGERATLSC	RASQSVGTNLA	WYQQKPGQAPRLIIF	DASTRDT

FIG. 11A

13/56

FR3	CDR3	FR4	J gene	SEQ ID NO
GVPSRFSGSGSGTDFLTISLQPEDFVAYYC	QKYNAPRT	FGGGTKVEIKRT	JK1	82
.....T.....V.....	..G.....	JK4	83
.....P.....V.H.....	..G.....	JK4	84
..I.D.....R.E...F.V.S.	..Q.GTS.W.	JK1	85
..I.D.....A.....I.....	..Q.H.S.Y.L.....	JK2	86
GIPDRFSGSGSGTDFLTISRLEPDDFVAYYC	QQYGDSPLYS	FGGGTKLEIKRT	JK2	87
GVPSRFSGRSGTDFLTISLQPEDFATYYC	QQGNSEPK	FGPGTVVDIKR	JK3	88
GIPDRFSGSGSGTDFLTISRLEPEDFVAYYC	QHYGNSVYT	FGGGTKLEIKR	JK2	89
GVPDRFSGSGSGTDFLTISRLEPEDFAMYYC	QYGGSSYT	FGGGTKVEITR	JK2	90
DIPDRFSGSGSGTDFLTISRLEPEDFATYYC	QVYGGSPVL	FGGGTKLEMR	JK2	91
GIPDRFSGSVSGTDFLTISRLEPEDFVAYYC	QRFGTSPLYT	FGGGTKLEMR	JK2	92
GVPDRFSGSGSGTDFLTISRLEPEDFVAYYC	QQYGASLVS	FGPGTKVHIKR	JK3	93
GISDRFSGSGSGTDFLTITRVEPEDFALYYC	QVYGASSYT	FGGGTKLERKRT	JK2	94
..P.....S.L.....V...	..Q.S.R..I....	JK2	95
.....L.....DF...	JK2	96
GIPDRFSGSGSGTDFLTISRLEPEDFVAYYC	QQYGTSPYT	FGGGTQLDIKRT	JK2	97
.....G.....T.NS.Q...V.T.S.	...N.V...K.E...	JK1	98
..G.S.....T.NS.Q...V.T.S.	..N.SA.W.KV....	JK1	99
GVPSRFSGSGSGTDFLTISLQPEDFATYYC	QQSYSIPPLT	FGGGTKVEIKRT	JK4	100
.....TA...E...I.R.....I.YT	..Q.....	JK2	101
.....I.....I.YT	..Q...L.....	JK2	102
.....I.....I.QT	..Q...L.....	JK2	103
.....G.....I.....I.YT	..Q...L.....	JK2	104
.....R...FI...V	..HQNV.LI	..Q...L.....	JK2	105
.....FT.....I.....	..YDNL.LI	JK4	106
.....I.....F.NT...WT	..Q.....	JK4	107
GVPDRFSGSGSGTDFLTISRLEPEDFVAYYC	QQFGDAQYT	FGGGTKLEIKRT	JK1	108
.....	JK2	109
GVPSRFSGSGSATYFSFTISLQPEDIGTYYC	QQYADLIT	FGGGTKVEIKRT	JK2	110
YIPDTFSGSGSGTDFALTISLQSEDFGFYYC	QQYDNWPPT	FGGGTKLEVKRT	JK4	111
			JK2	112
			JK2	113

FIG.11B

14/56

Clone	FR1	CDR1	FR2	CDR2
HIV-H12/L12	ELTQAPGTLSPGERATFSC	RSSHISRRVR	WYQHKPGQAPRLVIH	GVSNRAS
HIV-H12/LC11S.....D.....N.....A
HIV-H12/LC24S.....D.....N.....A
HIV-H12/LC22S.....N.....AT
HIV-H12/LC1S.D....NV....L..	.A..R.S...LAR.....L.Y	...S..G
HIV-H12/LC7S.D....NA....L..	.A..R.S...LAR.....L.YG
HIV-H12/LC28S.D....NT....L..	.A..R.G...LARR.....L.YG
HIV-H12/LC13S.....T.....IL..	KT..N.W...LAL.S.....L.Y	...K..G
HIV-H12/LC3S.....T.....IL..	KT..N.W...LAL.S.....L.Y	...K..G
HIV-H12/LC5S.....T.....IL..	KT..N.W...LAL.S.....L.Y	...K..G
HIV-H12/LC26S.....ST.....IL..	KT..N.W...LAV.S.LP...L..	...R..G
HIV-H12/LC25S.....N.....VL..	.T.RN.W...LAVRR.....L..	...K..G
HIV-H12/L12	ELTQAPGTLSPGERATFSC	RSSHISRRVR	WYQHKPGQAPRLVIH	GVSNRAS

FIG.12A

15/56

FR3	CDR3	FR4	SEQ ID NO
GISDRFSGSGGTDFTLTITRVEPEDFALYYC	QVYGASSYT	FGQGTKLERKR	97
.....L.....DF..	114
.....L.....DF..	114
.....L.....DF..	115
.VP.....S...S.L.....M...	.T..G....VDI..	116
.VP.....S...S.L.....I...	.T..G....TVDI..	117
.VP.....S...S.L.....I...	.T..G....VDI..	118
..P.....A.....S.....V....	.T..G.A...DI..	119
..P.....A.....S.....V....	.T..G.A...DI..	119
..P.....A.....S.....V....	.T..G.A...I...	120
..P.....AR.....S.L...A...V....	.T..G....SDFN.	121
.VP.....AR.....S.L.....V.F.	.T..G....N..DIR.	122
GISDRFSGSGGTDFTLTITRVEPEDFALYYC	QVYGASSYT	FGQGTKLERKR	97

FIG. 12B

16/56

FR1		CDR1	FR2	CDR2
H12/L12	LEQSGAEVKKPGASVKVSCQASGYRFS	NFVIH	WVRQAPGQRFWMG	WINPYNGNKEFSAKFQD
HC1	QVKL.....	...L.	.A.....H.P....A..VT.IPP....
HC2	QVKL.....	...L.	.A.....H.P....A..VT.I.P....
HC3	QVKL.....	...L.	.A.....H.P....A..VT.I.P....
HC7	QVKL.....I.....T	...L.	.A.....P....	.F..A..I..I.P....
HC9	QVKL.....	...L.	.A.....H.P....A..VT.I.P....
HC10	QVKL.....L.....	...L.	.A.....H.P....A..VT.I.P....
HC11	QVKL.....T.....I..K.....T	...PL.P....A..VT.I.P....
HC12	QVKL.....R.A..VT.I.P....
HC13	QVKL.....	...L.	.A....T.DL....A..V..I.P....
HC14	QVKL.....	...L.	.A.....H.P....A..VT.IPP....
H12-L12	LEQSGAEVKKPGASVKVSCQASGYRFS	NFVIH	WVRQAPGQRFWMG	WINPYNGNKEFSAKFQD

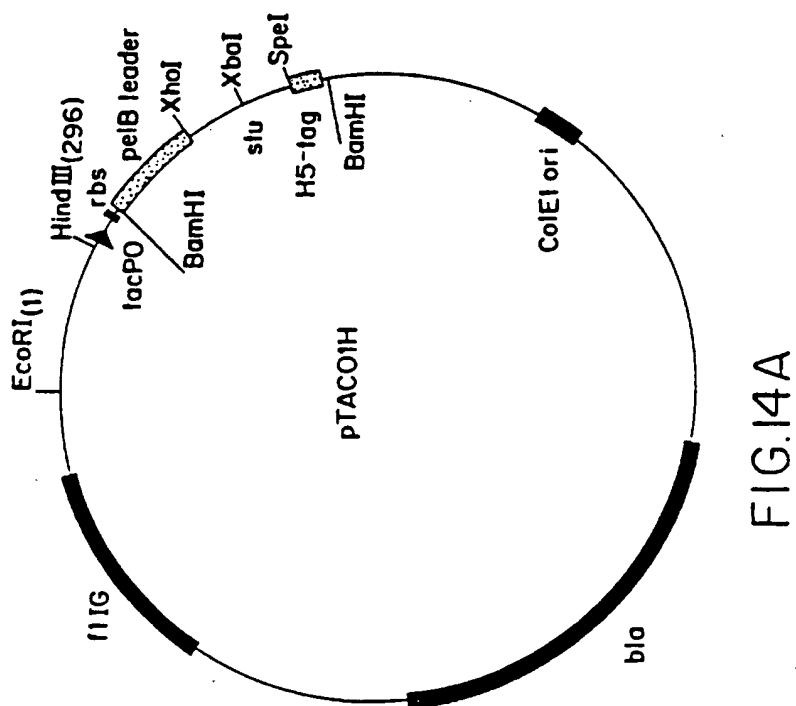
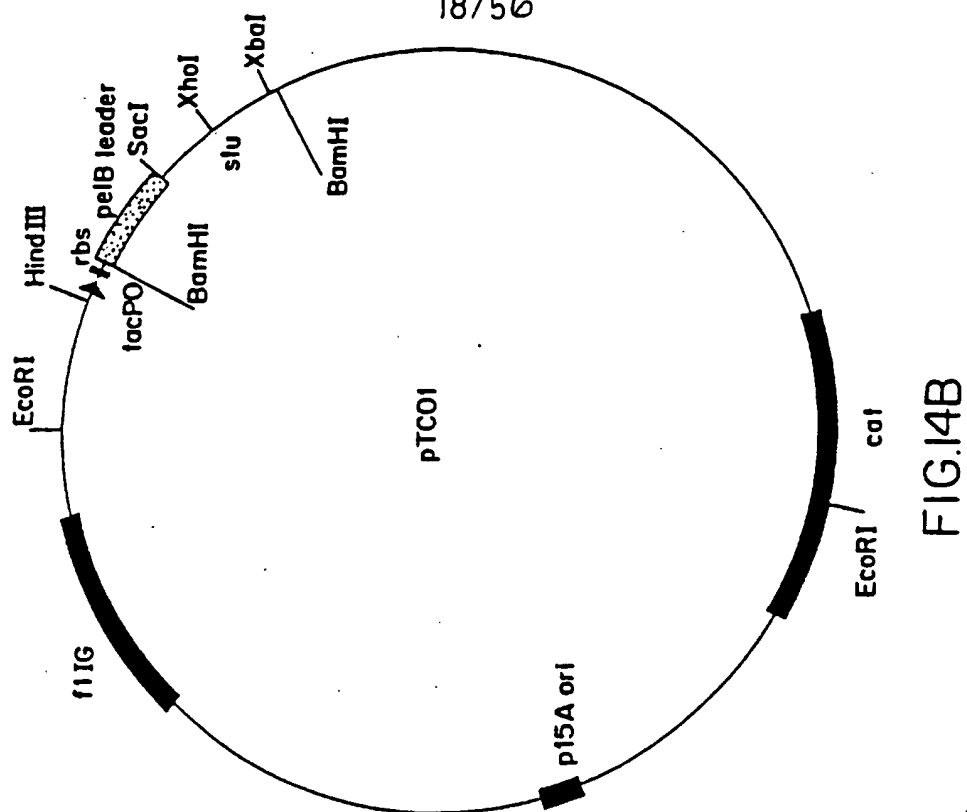
FIG. 13A

17/56

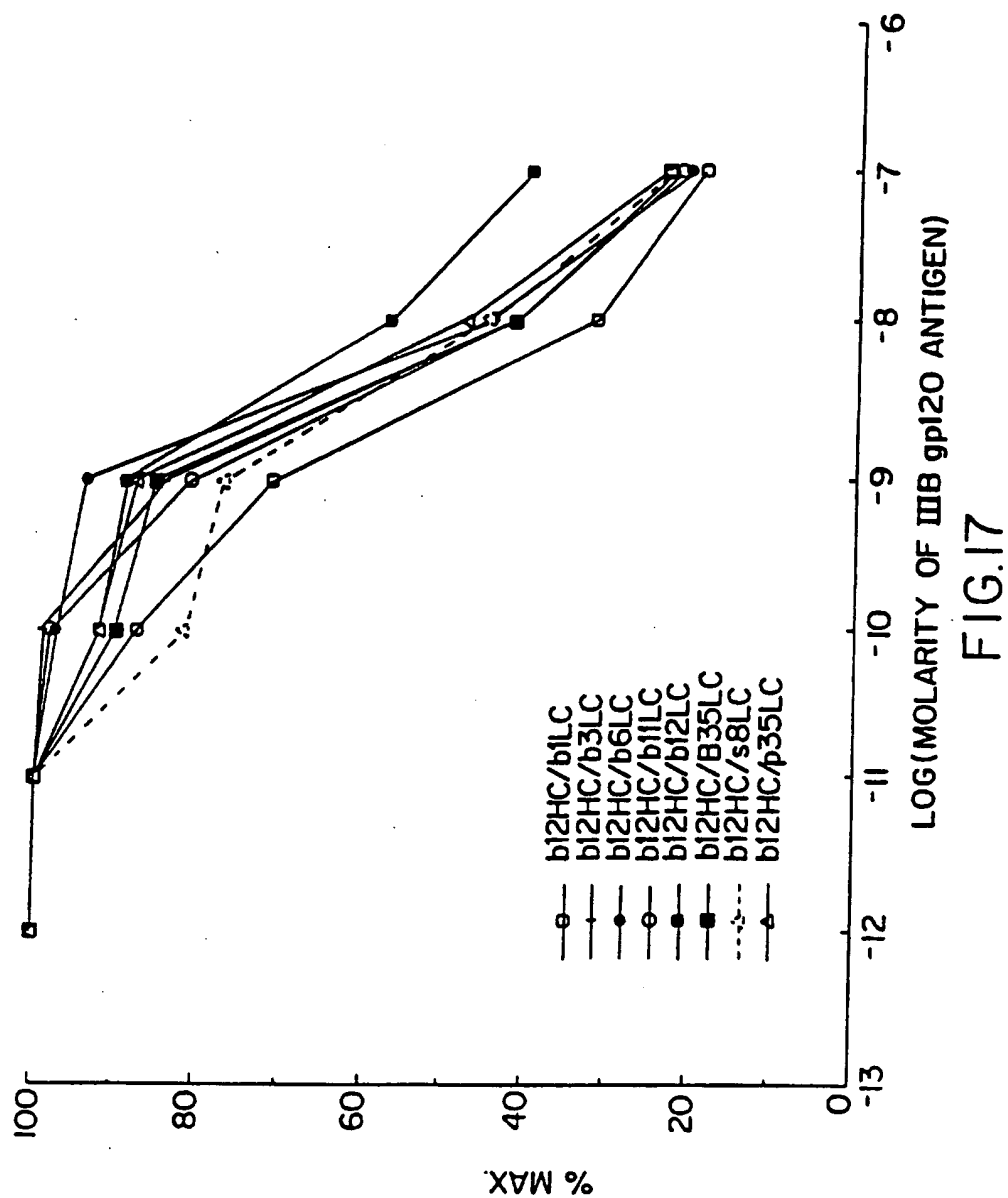
FR3	CDR3	FR4	SEQ ID NO
RVTFTADTSANTAYMELRSLRSADTAVYYCAR	VG PYSWDDSPQDNYYMDV	WGKGTTIVSS	66
..SL.R....G.V.L..TN..F.....	..EWT.....T.	123
..SL.G....S.V.L...N..F.....	..EWT.....	..R....T	124
..SL.G....S.V.L...F.....	..EWT.....	125
..S..G....S...V...N.....	..PWT.....	126
..SL.G....S.V.L...N..F.....	..EWT...F.....	127
..SL.G....S.V.L...N..F.....	..EWT.....T	128
....T.....V.G.....T.....	..EWT..MD..A.....T	129
.....D.....I.....T.....	130
..SL.G....S.V.L...F.....	..EWT.....	131
..SL.R....G.V.L..TN..F.....	..EWT.....	132
RVTFTADTSANTAYMELRSLRSADTAVYYCAR	VG PYSWDDSPQDNYYMDV	WGKGTTIVSS	66

FIG. 13B

18/56



21/56



22/56

CLONE	FR1	CDR1	FR2	CDR2
DL 41 19	LLESGPGLVKPSETLSLTCTVSGGSL	SFDWN	WIRQAPGKGLEWIG	RIYPSGNTHYNPSLRS
DO 41 11	LLKSGGGLVKPGGSLRLSCVISAFSFS	GYNIN	WVRQAPGKGLEWVS	SISMSTGSLSYADSMKG
GL 41 1	LLESGGGLVKPGGSLRLSCSASGFTFS	SYGMN	WVRQAPGKGPENVA	YISSSRKYTEYADSVKG
MT 41 12	LLEQSGGGLVQPGGSLRISCVASGDIIFYSYAMS		WVRQAPGKGLEWVA	SISGTGGSNYYADSVKG
SS 41 8	LLESGGGLVQPGGSLRLSCAASGFLYS	SFAMS	WVRQAPGKGLAWVS	TISASGGSTKYADSVKG

FIG. 18A

FR3	CDR3	FR4
RVTMSRDTSKNQFSVKLTSVTAADTALYYCAR	ENTGRTIEEIGNFFDI	WGQGTLVTVSSASTKG
RFTISRDNKNSVYLEMSSLTAEDTAMYCAA	RTPLVGRALDI	WGQGTVTVTVSSASTKG
RFTISRDNKNSVFLQLDSLTAEDTAIYYCAR	GRDFYSGFGRDRDDFHLHYMDV	WGKGTTVTVSSASTKG
RFTISRDNKNSKSTLYLQMNLSLRAEDTALYYCAR	DRGPRIGIRGWFD	WGQGTLVTVSSASTKG
RFTISRDNKNTIYLQMDLSLRAEDTAVYYCAK	NFAFARDPWGD	WGQGTLVTVSSASASTK

FIG. 18B

23/56

CLONE	FR1	CDR1	FR2	CDR2
DL 41 19	MAELTQSPGTLSPGERVIVSC	RASQSVSSNYLA	WYQKPGQAPRLLIY	GASNRAT
DO 41 11	MAELTQSPGTLSPGERATFSC	RSSHSHIRRA	WYQKPGQAPRLVIH	GVSNRAS
GL 41 1	MAELTQSPGTLSPGERATLSC	RASQSVSNGYLA	WYQKPGQAPRLLIY	GASTRAT
MT 41 12	MAELTQSPSSLSASVGDRVTITC	RPSQIGIRFFN	WYQKPGKAPNLLIY	AADILQS
SS 41 8	MAELTQSPSSLSASVGDRVTITC	RASQGVSSSYLA	WYQKPGQAPRLVIF	GAYS RAT

FIG. 19A

FR3	CDR3	FR4
GIPDRFSGSGGTDFTLTISRLEPEDFAVYYC	QQYGSSGT	FGQGTKVEIKRT
GISDRFSGSGGTDFTLTITRVEPEDFALYYC	QVYGASSYT	FGQGTKLERKRTV
DIPDRFSGSGGADFTLAIISRLEPEDFAVYYC	QQYAGSHT	FGQGTKLEIKRTVA
GVPSRFSGSGGTDFTLTISLQPEDFATYYC	QQSYSTPYT	FGQGTRLDIKRTVA
GIPDRFSGSGGTDFTLTISRLEPEDFAVYYC	QQYGSSPIT	FGPGTKVDIKRTVA

FIG. 19B

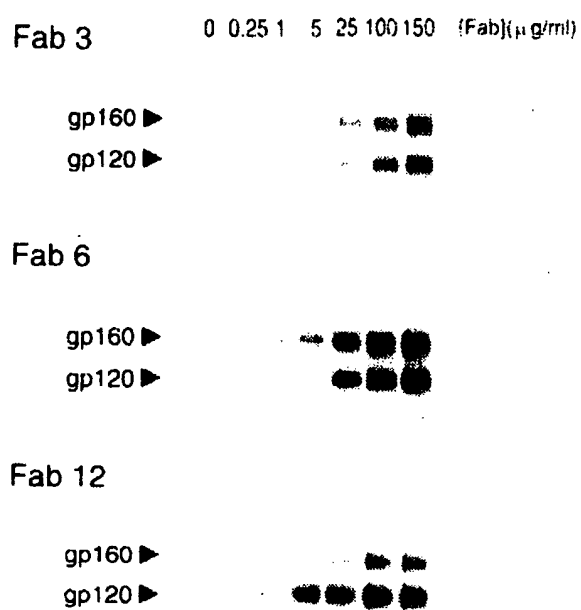


FIG. 20

25/56

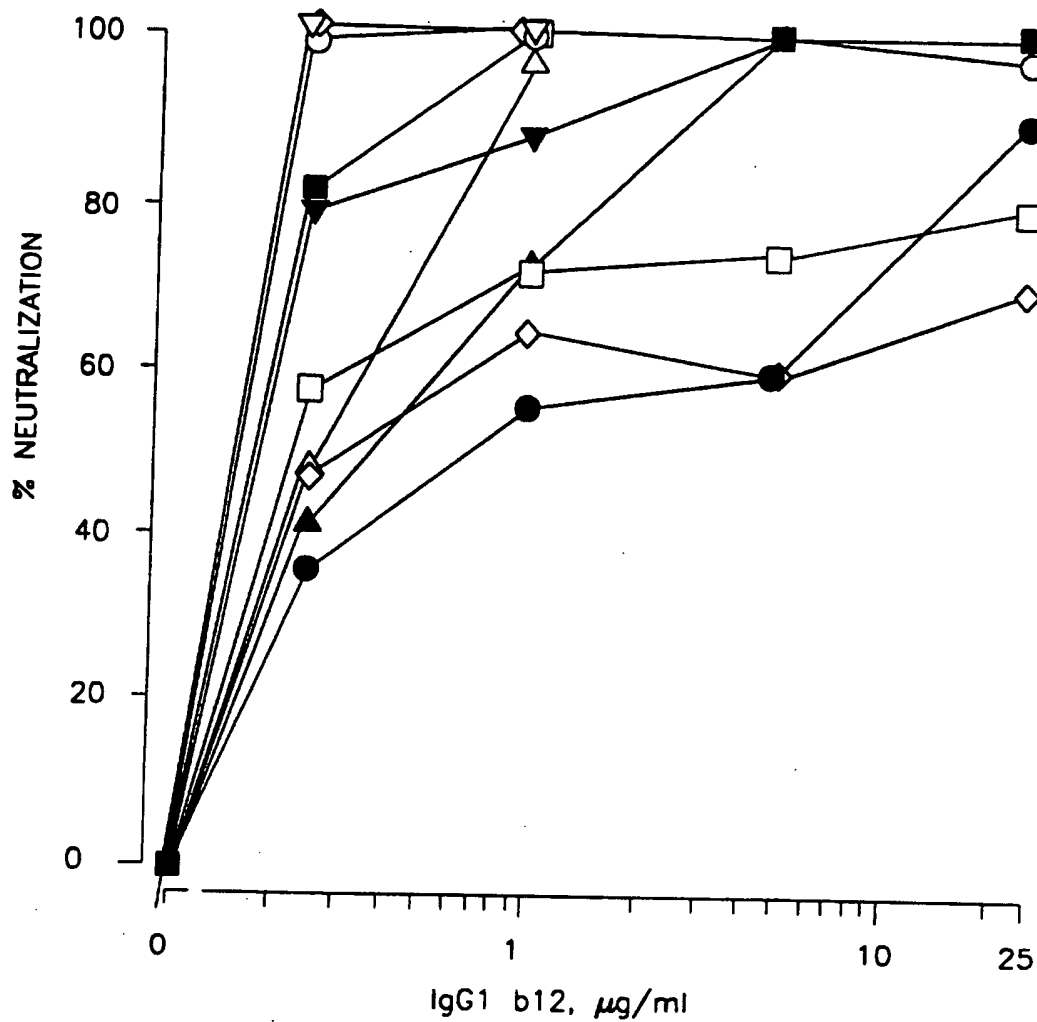


FIG. 21

26/56

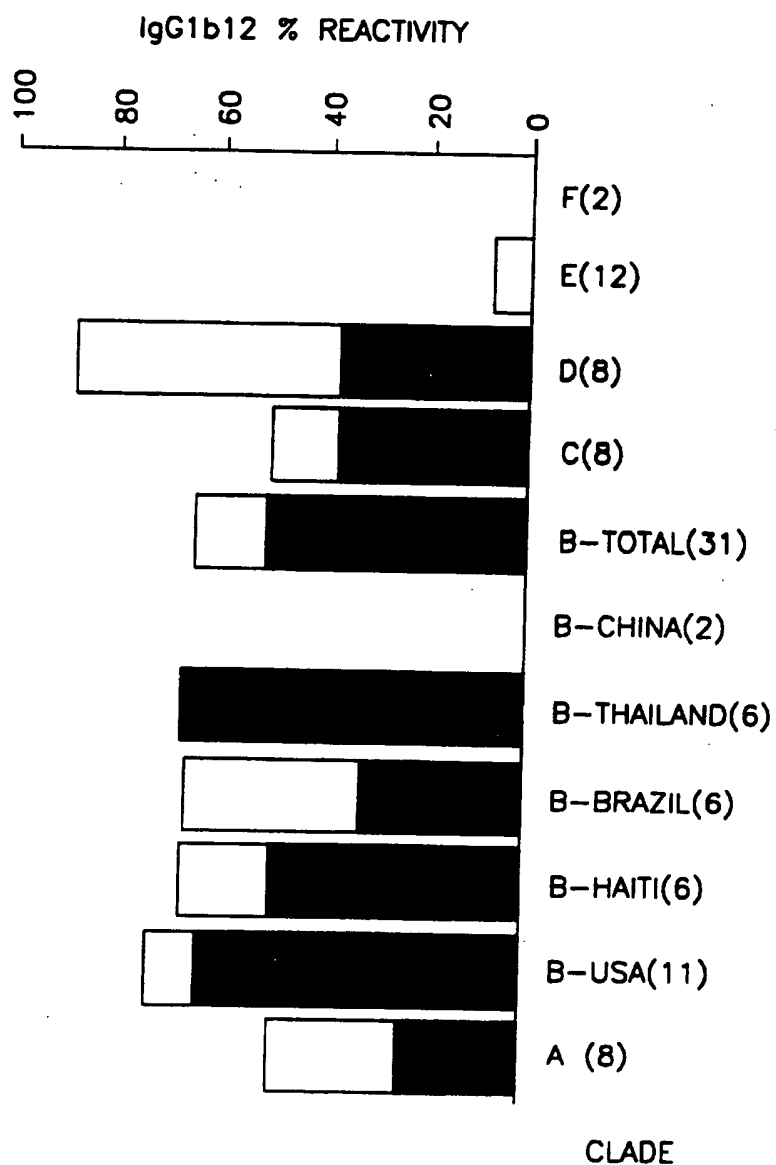


FIG. 22

27/5 6

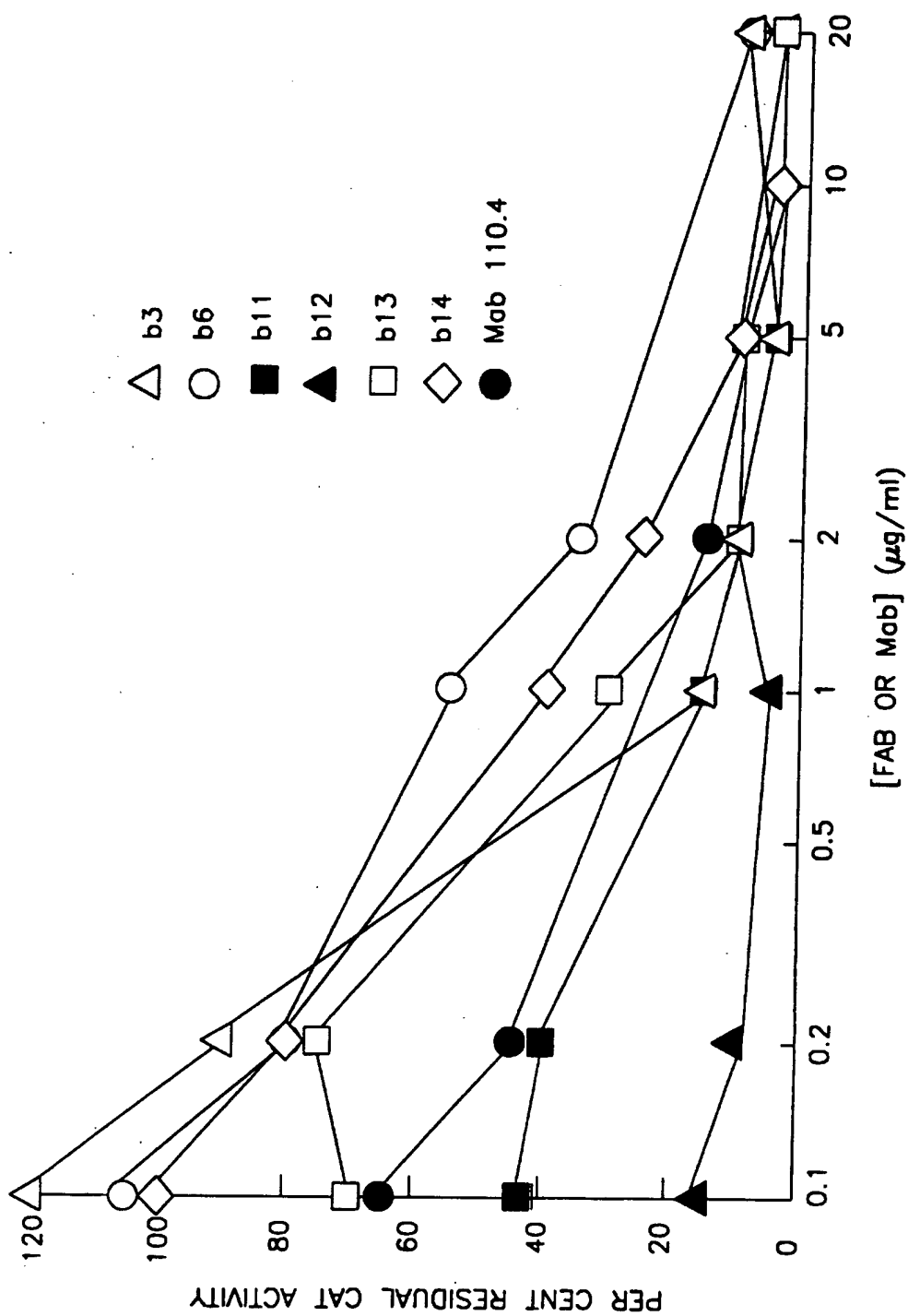


FIG. 23

28/56

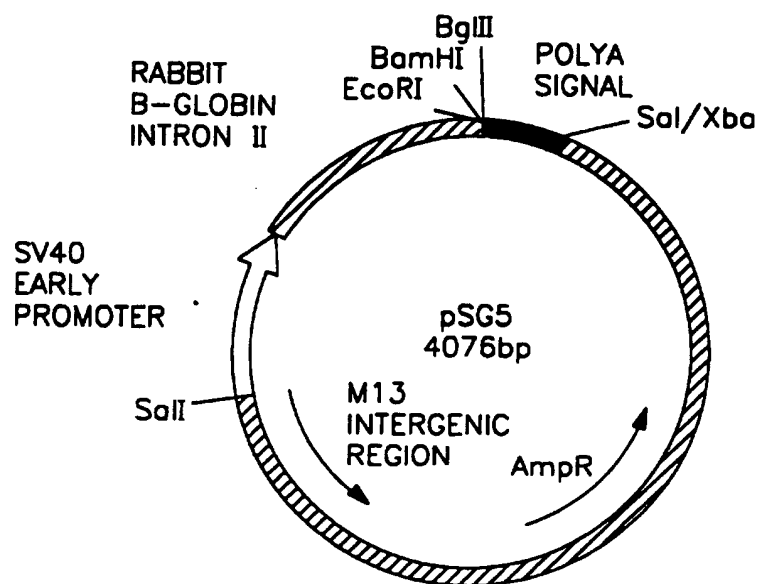


FIG. 24

29/56

5	10	15	20	25	30	35	40	45	50	55	60
	*		*		*		*		*		*
AGCTTACCAT	GGGTGTGCC	ACTCAGGTCC	TGGGGTTGCT	GCTGCTGTGG	CTTACAGATG						
TCGAATGGTA	CCCACACGGG	TGAGTCCAGG	ACCCCAACGA	CGACGACACC	GAATGTCTAC						
M	G V P	T Q V	L G L L	L L W	L T D>						
65	70	75	80	85	90	95	100	105	110	115	120
	*		*		*		*		*		*
CCAGATGTGA	GATCGTTCTC	ACGCAGTCTC	CAGGCACCCT	GTCTCTGTCT	CCAGGGGAAA						
GGTCTACACT	CTAGCAAGAG	TGCGTCAGAG	GTCCGTGGGA	CAGAGACAGA	GGTCCCCTTT						
A R C E	I V L	T Q S	P G T L	S L S	P G E>						
125	130	135	140	145	150	155	160	165	170	175	180
	*		*		*		*		*		*
GAGCCACCTT	CTCCTGTAGG	TCCAGTCACA	GCATTGCGAG	CCGCCGCGTA	GCCTGGTACC						
CTCGGTGGAA	GAGGACATCC	AGGTCAGTGT	CGTAAGCGTC	GGCGGCGCAT	CGGACCATGG						
R A T F	S C R	S S H	S I R S	R R V	A W Y>						
185	190	195	200	205	210	215	220	225	230	235	240
	*		*		*		*		*		*
AGCACAAACC	TGGCCAGGCT	CCAAGGCTGG	TCATACATGG	TGTTTCCAAT	AGGGCCTCTG						
TCGTGTTTGG	ACCGGTCCGA	GGTCCGACC	AGTATGTACC	ACAAAGGTTA	TCCCGGAGAC						
Q H K P	G Q A	P R L	V I H G	V S N	R A S>						
245	250	255	260	265	270	275	280	285	290	295	300
	*		*		*		*		*		*
GCATCTCAGA	CAGGTCAGC	GGCAGTGGGT	CTGGGACAGA	CTTCACTCTC	ACCATCACCA						
CGTAGAGTCT	GTCCAAGTCG	CCGTCACCCA	GACCCTGTCT	GAAGTGAGAG	TGGTAGTGGT						
G I S D	R F S	G S G	S G T D	F T L	T I T>						
305	310	315	320	325	330	335	340	345	350	355	360
	*		*		*		*		*		*
GAGTGGAGCC	TGAAGACTTT	GCACTGTACT	ACTGTCAGGT	CTATGGTGCC	TCCTCGTACA						
CTCACCTCGG	ACTTCTGAAA	CGTGACATGA	TGACAGTCCA	GATACCACGG	AGGAGCATGT						
R V E P	E D F	A L Y	Y C Q V	Y G A	S S Y>						
365	370	375	380	385	390	395	400	405	410	415	420
	*		*		*		*		*		*
CTTTTGGCCA	GGGGACCAAA	CTGGAGAGGA	AACGAACTGT	GCCTGCACCA	TCTGTCTTCA						
GAAAACCGGT	CCCCTGGTTT	GACCTCTCCT	TTGCTTGACA	CGGACGTGGT	AGACAGAAGT						
T F G Q	G T K	L E R	K R T V	P A P	S V F>						
425	430	435	440	445	450	455	460	465	470	475	480
	*		*		*		*		*		*
TCTTCCCGCC	ATCTGATGAG	CAGTTGAAAT	CTGGGACTGC	CTCTGTTGTG	TGCCTGCTGA						
AGAAGGGCGG	TAGACTACTC	GTCAACTTTA	GACCCTGACG	GAGACAACAC	ACGGACGACT						
I F P P	S D E	Q L K	S G T A	S V V	C L L>						
485	490	495	500	505	510	515	520	525	530	535	540
	*		*		*		*		*		*
ATAACTTCTA	TCCCAGAGAG	GCCAAAGTAC	AGTGGAAGGT	GGATAACGCC	CTCCAATCGG						
TATTGAAGAT	AGGGTCTCTC	CGGTTTCATG	TCACCTTCCA	CCTATTGCGG	GAGGTTAGCC						
N N F Y	P R E	A K V	Q W K V	D N A	L Q S>						
545	550	555	560	565	570	575	580	585	590	595	600
	*		*		*		*		*		*
GTAAGTCCCA	GGAGAGTGTC	ACAGAGCAGG	ACAGCAAGGA	CAGCACCTAC	AGCCTCAGCA						
CATTGAGGGT	CCTCTCACAG	TGTCTCGTCC	TGTCGTTTCT	GTCGTGGATG	TGGGAGTCGT						
G N S Q	E S V	T E Q	D S K D	S T Y	S L S>						

FIG. 25A

SUBSTITUTE SHEET (RULE 26)

30/56

605	610	615	620	625	630	635	640	645	650	655	660
*	*	*	*	*	*	*	*	*	*	*	*
GCACCCTGAC	GCTGAGCAAA	GCAGACTACG	AGAAACACAA	AGTCTACGCC	TGCGAAGTCA						
CGTGGGACTG	CGACTCGTTT	CGTCTGATGC	TCTTTGTGTT	TCAGATGCGG	ACGCTTCAGT						
S T L T	L S K	A D Y	E K H K	V Y A	C E V>						
665	670	675	680	685	690	695	700	705	710	715	720
*	*	*	*	*	*	*	*	*	*	*	*
CCCATCAGGG	CCTGAGTTTC	CCCGTCACAA	AGAGCTTCAA	CAGGGGAGAG	TGTTAATTCT						
GGGTAGTCCC	GGACTCAAGC	GGGCAGTGT	TCTCGAAGTT	GTCCCCTCTC	ACAATTAAGA						
T H Q G	L S S	P V T	K S F N	R G E	C *>						

725
AGAGAATTC
TCTCTTAAG

FIG. 25B

31/56

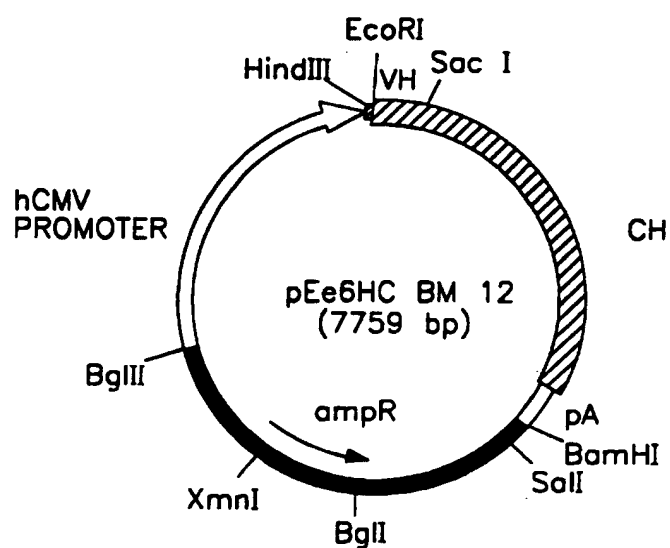


FIG. 26

32/56

5	10	15	20	25	30	35	40	45	50	55	60
	*		*		*		*		*		*
AATTCGCCGC	CACCATGGAA	TGGAGCTGGG	TCTTTCTCTT	CTTCCTGTCA	GTAACACAG						
TTAAGCGGCG	GTGGTACCTT	ACCTCGACCC	AGAAAGAGAA	GAAGGACAGT	CATTGATGTC						
	M E	W S W	V F L F	F L S	V T T>						
65	70	75	80	85	90	95	100	105	110	115	120
	*		*		*		*		*		*
GTGTCCACTC	CCAGGTTTCA	CTGGTTTCA	CTGGTTTCA	CTGGTTTCA	CTGGTTTCA	CTGGTTTCA	CTGGTTTCA	CTGGTTTCA	CTGGTTTCA	CTGGTTTCA	CTGGTTTCA
CACAGGTGAG	GGTCCAAGTC	GACCAAGTCA	GGCCCCGACT	CCACTTCTTC	GGACCCCGGA						
G V H S	Q V Q	L V Q	S G A E	V K K	P G A>						
125	130	135	140	145	150	155	160	165	170	175	180
	*		*		*		*		*		*
CAGTGAAGGT	TTCTTGTGAG	GCTTCTGGAT	ACAGATTCAG	TAACTTTGTT	ATTCATTGGG						
GTCACCTTCA	AAGAACAGTC	CGAAGACCTA	TGTCTAAGTC	ATTGAAACAA	TAAGTAACCC						
S V K V	S C Q	A S G	Y R F S	N F V	I H W>						
185	190	195	200	205	210	215	220	225	230	235	240
	*		*		*		*		*		*
TGCGCCAGGC	CCCCGGACAG	AGGTTTGA	GGATGGGATG	GATCAATCCT	TACAACGGAA						
ACGCGGTCCG	GGGGCCTGTC	TCCAAACTCA	CCTACCCTAC	CTAGTTAGGA	ATGTTGCCTT						
V R Q A	P G Q	R F E	W M G W	I N P	Y N G>						
245	250	255	260	265	270	275	280	285	290	295	300
	*		*		*		*		*		*
ACAAAGAATT	TTCAGCGAAG	TTCCAGGACA	GAGTCACCTT	TACCGCGGAC	ACATCCGCGA						
TGTTTCTTAA	AAGTCGCTTC	AAGTCCTGT	CTCAGTGGAA	ATGGCGCCTG	TGTAGGCGCT						
N K E F	S A K	F Q D	R V T F	T A D	T S A>						
305	310	315	320	325	330	335	340	345	350	355	360
	*		*		*		*		*		*
ACACAGCCTA	CATGGAGTTG	AGGAGCCTCA	GGTCTGCAGA	CACGGCTGTT	TATTATTGTG						
TGTGTCGGAT	GTACCTCAAC	TCCTCGGAGT	CCAGACGTCT	GTGCCGACAA	ATAATAACAC						
N T A Y	M E L	R S L	R S A D	T A V	Y Y C>						
365	370	375	380	385	390	395	400	405	410	415	420
	*		*		*		*		*		*
CGAGAGTGGG	GCCATATAGT	TGGGATGATT	CTCCCCAGGA	CAATTATTAT	ATGGACGTCT						
GCTCTCACCC	CGGTATATCA	ACCCTACTAA	GAGGGGTCCT	GTAAATAATA	TACCTGCAGA						
A R V G	P Y S	W D D	S P Q D	N Y Y	M D V>						
425	430	435	440	445	450	455	460	465	470	475	480
	*		*		*		*		*		*
GGGGCAAAGG	AACCACGGTC	ATCGTGAGCT	CAGCTTCCAC	CAAGGGCCCA	TCGGTCTTCC						
CCCCGTTTCC	TTGGTGCCAG	TAGCACTCGA	GTCGAAGGTG	GTTCCCGGGT	AGCCAGAAGG						
W G K G	T T V	I V S	S>								
485	490	495	500	505	510	515	520	525	530	535	540
	*		*		*		*		*		*
CCCTGGCACC	CTCCTCCAAG	AGCACCTCTG	GGGGCACAGC	GGCCCTGGGC	TGCCTGGTCA						
GGGACCGTGG	GAGGAGGTTT	TCGTGGAGAC	CCCCGTGTCT	CCGGGACCCG	ACGGACCACT						
545	550	555	560	565	570	575	580	585	590	595	600
	*		*		*		*		*		*
AGGACTACTT	CCCCGAACCG	GTGACGGTGT	CGTGGAACTC	AGGCGCCCTG	ACCAGCGGCG						
TCCTGATGAA	GGGGCTTGGC	CACTGCCACA	GCACCTTGAG	TCCGCGGGAC	TGGTCCGCCG						

FIG. 27A

SUBSTITUTE SHEET (RULE 26)

33/56

605	610	615	620	625	630	635	640	645	650	655	660
*	*	*	*	*	*	*	*	*	*	*	*
TGCACACCTT	CCCGGCTGTC	CTACAGTCCT	CAGGACTCTA	CTCCCTCAGC	AGCGTGGTGA						
ACGTGTGGAA	GGGCCGACAG	GATGTCAGGA	GTCCTGAGAT	GAGGGAGTCG	TCCGACCACT						
665	670	675	680	685	690	695	700	705	710	715	720
*	*	*	*	*	*	*	*	*	*	*	*
CCGTGCCCTC	CAGCAGCTTG	GGCACCCAGA	CCTACATCTG	CAACGTGAAT	CACAAGCCCA						
GGCACGGGAG	GTCGTCGAAC	CCGTGGGTCT	GGATGTAGAC	GTTGCACTTA	GTGTTCCGGT						
725	730	735	740	745	750	755	760	765	770	775	780
*	*	*	*	*	*	*	*	*	*	*	*
GCAACACCAA	GGTGGACAAG	AAAGTTGGTG	AGAGGCCAGC	ACAGGGAGGG	AGGGTGTCTG						
CGTTGTGGTT	CCACCTGTTC	TTTCAACCAC	TCTCCGGTCG	TGTCCCTCCC	TCCCACAGAC						
785	790	795	800	805	810	815	820	825	830	835	840
*	*	*	*	*	*	*	*	*	*	*	*
CTGGAAGCCA	GGCTCAGCGC	TCCTGCCTGG	ACGCATCCCG	GCTATGCAGC	CCCAGTCCCA						
GACCTTCGGT	CCGAGTCGCG	AGGACGGACC	TGCGTAGGGC	CGATACGTCG	GGGTGAGGTC						
845	850	855	860	865	870	875	880	885	890	895	900
*	*	*	*	*	*	*	*	*	*	*	*
GGCAGCAAGG	CAGGCCCGCT	CTGCCTCTTC	ACCCGGAGGC	CTCTGCCCGC	CCCACTCATG						
CCGTTCGTTCC	GTCCGGGGCA	GACGGAGAAG	TGGGCCTCCG	GAGACGGGCG	GGGTGAGTAC						
905	910	915	920	925	930	935	940	945	950	955	960
*	*	*	*	*	*	*	*	*	*	*	*
CTCAGGGAGA	GGGTCTTCTG	GCTTTTTCCT	CAGGCTCTGG	GCAGGCACAG	GCTAGGTGCC						
GAGTCCCTCT	CCCAGAAGAC	CGAAAAAGGG	GTCCGAGACC	CGTCCGTGTC	CGATCCACGG						
965	970	975	980	985	990	995	1000	1005	1010	1015	1020
*	*	*	*	*	*	*	*	*	*	*	*
CCTAACCCAG	GCCCTGCACA	CAAAGGGGCA	GGTGCTGGGC	TCAGACCTGC	CAAGAGCCAT						
GGATTGGGTC	CGGGACGTGT	GTTTCCCCGT	CCACGACCCG	AGTCTGGACC	GTTCTCGGTA						
1025	1030	1035	1040	1045	1050	1055	1060	1065	1070	1075	1080
*	*	*	*	*	*	*	*	*	*	*	*
ATCCGGGAGG	ACCCTGCCCC	TGACCTAAGC	CCACCCCAAA	GGCCAAATC	TCCACTCCCT						
TAGGCCCTCC	TGGGACGGGG	ACTGGATTCC	GGTGGGGTTT	CCGGTTTGTG	AGGTGAGGGA						
1085	1090	1095	1100	1105	1110	1115	1120	1125	1130	1135	1140
*	*	*	*	*	*	*	*	*	*	*	*
CAGCTCGGAC	ACCTTCTCTC	CTCCCAGATT	CGAGTAACTC	CCAATCTTCT	CTCTGCAGAG						
GTCGAGCCTG	TGGAAGAGAG	GAGGGTCTAA	GCTCATTGAG	GGTTAGAAGA	GAGACGTCTC						
1145	1150	1155	1160	1165	1170	1175	1180	1185	1190	1195	1200
*	*	*	*	*	*	*	*	*	*	*	*
CCCAAATCTT	GTGACAAAAC	TCACACATGC	CCACCGTGCC	CAGGTAAGCC	AGCCCAGGCC						
GGGTTTAGAA	CACTGTTTTG	AGTGTGTACG	GGTGGCACGG	GTCCATTCCG	TCGGGTCCGG						
1205	1210	1215	1220	1225	1230	1235	1240	1245	1250	1255	1260
*	*	*	*	*	*	*	*	*	*	*	*
TCGCCCTCCA	GCTCAAGGCG	GGACAGGTGC	CCTAGAGTAG	CCTGCATCCA	GGGACAGGCC						
AGCGGGAGGT	CGAGTTCGCG	CCTGTCCACG	GGATCTCATC	GGACGTAGGT	CCCTGTCCGG						
1265	1270	1275	1280	1285	1290	1295	1300	1305	1310	1315	1320
*	*	*	*	*	*	*	*	*	*	*	*
CCAGCCGGGT	GCTGACACGT	CCACCTCCAT	CTCTCCCTCA	GCACCTGAGG	CCGCGGGAGG						
GGTCGGCCCA	CGACTGTGCA	GGTGGAGGTA	GAGAGGGAGT	CGTGGACTCC	GGCGCCCTCC						

FIG. 27B

SUBSTITUTE SHEET (RULE 26)

34/56

1325 1330	1335 1340	1345 1350	1355 1360	1365 1370	1375 1380
* ACCATCAGTC	* TTCCTCTTCC	* CCCCAAAACC	* CAAGGACACC	* CTCATGATCT	* CCCGGACCCC
TGGTAGTCAG	AAGGAGAAGG	GGGGTTTTGG	GTTCTGTGG	GAGTACTAGA	GGGCCTGGGG
1385 1390	1395 1400	1405 1410	1415 1420	1425 1430	1435 1440
* TGAGGTCACA	* TGCCTGGTGG	* TGGACGTGAG	* CCACGAAGAC	* CCTGAGGTCA	* AGTTCAACTG
ACTCCAGTGT	ACGCACCACC	ACCTGCACTC	GGTGCTTCTG	GGACTCCAGT	TCAAGTTGAC
1445 1450	1455 1460	1465 1470	1475 1480	1485 1490	1495 1500
* GTACGTGGAC	* GGCGTGGAGG	* TGCATAATGC	* CAAGACAAAG	* CCGCGGGAGG	* AGCAGTACAA
CATGCACCTG	CCGCACCTCC	ACGTATTACG	GTTCTGTTTC	GGCGCCCTCC	TCGTCATGTT
1505 1510	1515 1520	1525 1530	1535 1540	1545 1550	1555 1560
* CAGCACGTAC	* CGTGTGGTCA	* GCGTCCTCAC	* CGTCCTGCAC	* CAGGACTGGC	* TGAATGGCAA
GTCTGTCATG	GCACACCAGT	CGCAGGAGTG	GCAGGACGTG	GTCTTGACCG	ACTTACCCTT
1565 1570	1575 1580	1585 1590	1595 1600	1605 1610	1615 1620
* GGAGTACAAG	* TGCAAGGTCT	* CCAACAAAGC	* CCTCCCAGCC	* CCCATCGAGA	* AAACCATCTC
CCTCATGTTC	ACGTTCCAGA	GGTTGTTTCG	GGAGGCTCGG	GGGTAGCTCT	TTTGTTAGAG
1625 1630	1635 1640	1645 1650	1655 1660	1665 1670	1675 1680
* CAAAGCCAAA	* GGTGGGACCC	* GTGGGGTGCG	* AGGGCCACAT	* GGACAGAGGC	* CGGCTCGGCC
GTTTCGGTTT	CCACCCTGGG	CACCCCACGC	TCCCGGTGTA	CCTGTCTCCG	GCCGAGCCGG
1685 1690	1695 1700	1705 1710	1715 1720	1725 1730	1735 1740
* CACCTCTGCG	* CCTGAGAGTG	* ACCGCTGTAC	* CAACCTCTGT	* CCCTACAGGG	* CAGCCCCGAG
GTGGGAGACG	GGACTCTCAC	TGGCGACATG	GTTGGAGACA	GGGATGTCCC	GTCGGGGCTC
1745 1750	1755 1760	1765 1770	1775 1780	1785 1790	1795 1800
* AACCACAGGT	* GTACACCCTG	* CCCCCATCCC	* GGGATGAGCT	* GACCAAGAAC	* CAGGTCAGCC
TTGGTGTCCA	CATGTGGGAC	GGGGGTAGGG	CCCTACTCGA	CTGGTTCTTG	GTCCAGTCGG
1805 1810	1815 1820	1825 1830	1835 1840	1845 1850	1855 1860
* TGACCTGCCT	* GGTCAAAGGC	* TTCTATCCCA	* GCGACATCGC	* CGTGGAGTGG	* GAGAGCAATG
ACTGGACGGA	CCAGTTTCCG	AAGATAGGGT	CGCTGTAGCG	GCACCTCACC	CTCTCGTTAC
1865 1870	1875 1880	1885 1890	1895 1900	1905 1910	1915 1920
* GGCAGCCGGA	* GAACAACTAC	* AAGACCACGC	* CTCCCGTGCT	* GGACTCCGAC	* GGCTCCTTCT
CCGTCGGCCT	CTTGTTGATG	TCTGGTGCG	GAGGGCACGA	CCTGAGGCTG	CCGAGGAAGA
1925 1930	1935 1940	1945 1950	1955 1960	1965 1970	1975 1980
* TCCTCTACAG	* CAAGCTCACC	* GTGGACAAGA	* GCAGGTGGCA	* GCAGGGGAAC	* GTCTTCTCAT
AGGAGATGTC	GTTTCGAGTG	CACCTGTTCT	CGTCCACCGT	CGTCCCCTTG	CAGAAGAGTA
1985 1990	1995 2000	2005 2010	2015 2020	2025 2030	2035 2040
* GCTCCGTGAT	* GCATGAGGCT	* CTGCACAACC	* ACTACACGCA	* GAAGAGCCTC	* TCCCTGTCTC
CGAGGCACTA	CGTACTCCGA	GACGTGTTGG	TGATGTGCGT	CTTCTCGGAG	AGGGACAGAG

FIG. 27C

SUBSTITUTE SHEET (RULE 5)

35/56

2045	2050	2055	2060	2065	2070	2075	2080	2085	2090	2095	2100
CGGGTAAATG	AGTGCGACGG	CCGGCAAGCC	CCCGCTCCCC	GGGCTCTCGC	GGTTCGCACGA	GGCCATTTAC	TCACGCTGCC	GGCCGTTCGG	GGGCGAGGGG	CCCAGAGCG	CCAGCGTGCT
2105	2110	2115	2120	2125	2130	2135	2140	2145	2150	2155	2160
GGATGCTTGG	CACGTACCCC	CTGTACATAC	TTCCCGGGCG	CCCAGCATGG	AAATAAAGCA	CCTACGAACC	GTGCATGGGG	GACATGTATG	AAGGGCCCCG	GGGTCGTACC	TTTATTTCTG
2165	2170	2175	2180	2185	2190	2195	2200	2205	2210	2215	2220
CCCAGCGCTG	CCCTGGGCCC	CTGCGAGACT	GTGATGGTTC	TTTCCACGGG	TCAGGCCGAG	GGGTCGCGAC	GGGACCCGGG	GACGCTCTGA	CACTACCAAG	AAAGGTGCCC	AGTCCGGCTC
2225	2230	2235	2240	2245	2250	2255	2260	2265	2270	2275	2280
TCTGAGGCCT	GAGTGGCATG	AGGGAGGCAG	AGCGGGTCCC	ACTGTCCCCA	CACTGGCCCCA	AGACTCCGGA	CTCACCGTAC	TCCCTCCGTC	TGCCCCAGGG	TGACAGGGGT	GTGACCGGGT
2285	2290	2295	2300	2305	2310	2315	2320	2325	2330	2335	2340
GGCTGTGCAG	GTGTGCCTGG	GCCGCCTAGG	GTGGGGCTCA	GCCAGGGGCT	GCCCTCGGCA	CCGACACGTC	CACACGGACC	CGGCGGATCC	CACCCCGAGT	CGGTCCCCGA	CGGGAGCCGT
2345	2350	2355	2360	2365	2370	2375	2380	2385	2390	2395	2400
GGGTGGGGGA	TTTGCCAGCG	TTGCCCTCCC	TCCAGCAGCA	CCTGCCCTGG	GCTGGGCCAC	CCCACCCCT	AAACGGTCGC	AACGGGAGGG	AGGTCTGCTG	GGACGGGACC	CGACCCGGTG
2405	2410	2415	2420	2425	2430	2435	2440	2445	2450	2455	2460
GGGAAGCCCT	AGGAGCCCCT	GGGGACAGAC	ACACAGCCCC	TGCCTCTGTA	GGAGACTGTC	CCCTTCGGGA	TCCTCGGGGA	CCCCTGTCTG	TGTGTCGGGG	ACGGAGACAT	CCTCTGACAG
2465	2470	2475	2480	2485	2490	2495	2500	2505	2510	2515	2520
CTGTTCTGTG	AGCGCCCTGT	CCTCCGACCT	CCATGCCAC	TCGGGGGCAT	GCCTAGTCCA	GACAAGACAC	TCGCGGGACA	GGAGGCTGGA	GGTACGGGTG	AGCCCCCGTA	CGGATCAGGT
2525	2530	2535	2540	2545	2550	2555	2560	2565	2570	2575	2580
TGTGCGTAGG	GACAGGCCCT	CCCTCACCCA	TCTACCCCCA	CGGCACTAAC	CCCTGGCTGT	ACACGCATCC	CTGTCCGGGA	GGGAGTGGGT	AGATGGGGGT	GCCGTGATTG	GGGACCGACA
2585	2590	2595	2600	2605	2610	2615	2620	2625	2630	2635	2640
CCTGCCCAGC	CTCGCACCCG	CATGGGGACA	CAACCGACTC	CGGGGACATG	CACTCTCGGG	GGACGGGTCTG	GAGCGTGGGC	GTACCCCTGT	GTGGCTGAG	GCCCCCTGTAC	GTGAGAGCCC
2645	2650	2655	2660	2665	2670	2675	2680	2685	2690	2695	2700
CCCTGTGGAG	GGACTGGTGC	AGATGCCAC	ACACACACTC	AGTCCAGACC	CGTTCAACAA	GGGACACCTC	CCTGACCACG	TCTACGGGTG	TGTGTGTGAG	TCAGGTCTGG	GCAAGTTGTT
2705	2710	2715	2720	2725	2730	2735	2740	2745	2750	2755	2760
AACCCCCGCA	CTGAGGTTGG	CCGGCCACAC	GGCCACCACA	CACACACGTG	CACGCCTCAC	TTGGGGGCGT	GACTCCAACC	GGCCGTGTG	CCGGTGGTGT	GTGTGTGCAC	GTGCGGAGTG

FIG. 27D

36/56

2765	2770	2775	2780	2785	2790	2795	2800	2805	2810	2815	2820
*	*	*	*	*	*	*	*	*	*	*	*
ACACGGAGCC	TCACCCGGGC	GAAGTGCACA	GCACCCAGAC	CAGAGCAAGG	TCCTCGCACA	TGTGCCTCGG	AGTGGGCCCC	CTTGACGTGT	CGTGGGTCTG	GTCTCGTTCC	AGGAGCGTGT
2825	2830	2835	2840	2845	2850	2855	2860	2865	2870	2875	2880
*	*	*	*	*	*	*	*	*	*	*	*
CGTGAACACT	CCTCGGACAC	AGGCCCCCAC	GAGCCCCACG	CGGCACCTCA	AGGCCCCACG	GCACTTGTGA	GGAGCCTGTG	TCCGGGGGTG	CTCGGGGTGC	GCCGTGGAGT	TCCGGGTGCT
2885	2890	2895	2900	2905	2910	2915	2920	2925	2930	2935	2940
*	*	*	*	*	*	*	*	*	*	*	*
GCCTCTCGGC	AGCTTCTCCA	CATGCTGACC	TGCTCAGACA	AACCCAGCCC	TCCTCTCACA	CGGAGAGCCG	TCGAAGAGGT	GTACGACTGG	ACGAGTCTGT	TTGGGTCTGGG	AGGAGAGTGT
2945	2950	2955	2960	2965	2970	2975	2980	2985	2990	2995	3000
*	*	*	*	*	*	*	*	*	*	*	*
AGGGTGCCCC	TGCAGCCGCC	ACACACACAC	AGGGGATCAC	ACACCACGTC	ACGTCCCTGG	TCCCACGGGG	ACGTCCGGCG	TGTGTGTGTG	TCCCCTAGTG	TGTGGTGCAG	TGCAGGGACC
3005	3010	3015	3020	3025	3030	3035	3040	3045	3050	3055	3060
*	*	*	*	*	*	*	*	*	*	*	*
CCCTGGCCCA	CTTCCAGTG	CCGCCCTTCC	CTGCAGGGCG	GATCATAATC	AGCCATACCA	GGGACCGGGT	GAAGGGTCAC	GGCGGGAAGG	GACGTCCCGC	CTAGTATTAG	TCGGTATGGT
3065	3070	3075	3080	3085	3090	3095	3100	3105	3110	3115	3120
*	*	*	*	*	*	*	*	*	*	*	*
CATTTGTAGA	GGTTTTACTT	GCTTTAAAAA	ACCTCCACAC	CCTCCCCCTG	AACCTGAAAC	GTAAACATCT	CCAAAATGAA	CGAAATTTT	TGGAGGGTGT	GGAGGGGGAC	TTGGACTTTG
3125	3130	3135	3140	3145	3150	3155	3160	3165	3170	3175	3180
*	*	*	*	*	*	*	*	*	*	*	*
ATAAAATGAA	TGCAATTGTT	GTTGTAACT	TGTTTATTGC	AGCTTATAAT	GGTTACAAAT	TATTTTACTT	ACGTTAACAA	CAACAATTGA	ACAAATAACG	TCGAATATTA	CCAATGTTTA
3185	3190	3195	3200	3205	3210	3215	3220	3225	3230	3235	3240
*	*	*	*	*	*	*	*	*	*	*	*
AAAGCAATAG	CATCACAAAT	TTCACAAATA	AAGCATTTTT	TTCAGTGCAT	TCTAGTTGTG	TTTCGTTATC	GTAGTGTITA	AAGTGTITAT	TTCGTAAAAA	AAGTGACGTA	AGATCAACAC
3245	3250	3255	3260	3265	3270	3275	3280				
*	*	*	*	*	*	*	*				
GTTTGTCCAA	ACTCATCAAT	GTATCTTATC	ATGTCTAGAT	CC							
CAAACAGGTT	TGAGTAGTTA	CATAGAATAG	TACAGATCTA	GG							

FIG. 27E

37/56

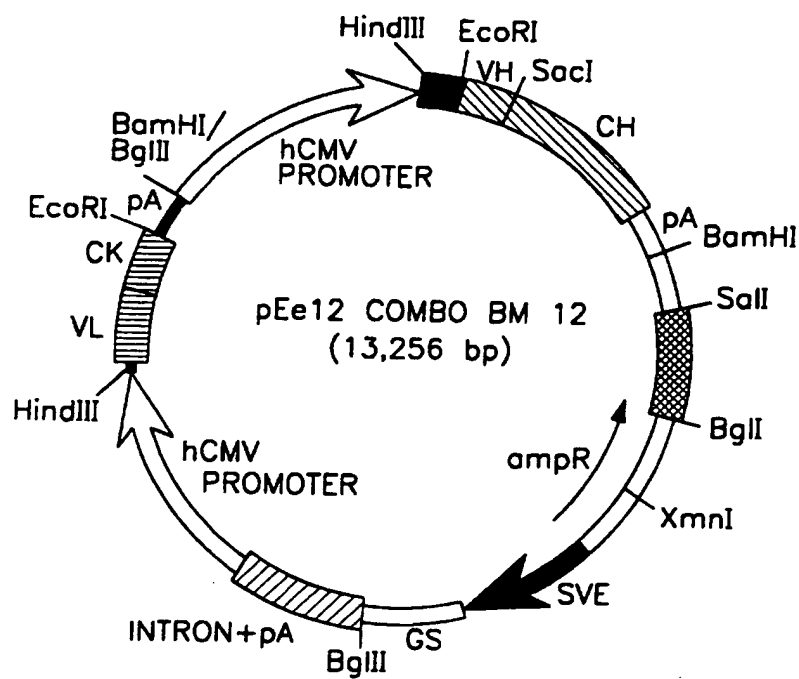


FIG. 28

38/56

5	10	15	20	25	30	35	40	45	50	55	60
TTCATTGATC	ATTAATCAGC	CATACCACAT	TTGTAGAGGT	TTTACTTGCT	TTAAAAAACC						
AAGTAACTAG	TAATTAGTCG	GTATGGTGTA	AACATCTCCA	AAATGAACGA	AATTTTTTGG						
65	70	75	80	85	90	95	100	105	110	115	120
TCCCACACCT	CCCCCTGAAC	CTGAAACATA	AAATGAATGC	AATTGTTGTT	GTAACTTGT						
AGGGTGTGGA	GGGGGACTTG	GACTTTGTAT	TTTACTTACG	TTAACAACAA	CAATTGAACA						
125	130	135	140	145	150	155	160	165	170	175	180
TTATTGCAGC	TTATAATGGT	TACAAATAAA	GCAATAGCAT	CACAAATTTT	ACAAATAAAG						
AATAACGTCTG	AATATTACCA	ATGTTTATTT	CGTTATCGTA	GTGTTTAAAG	TGTTTATTTT						
185	190	195	200	205	210	215	220	225	230	235	240
CATTTTTTTT	ACTGCATTCT	AGTTGTGGTT	TGTCCAAACT	CATCAATGTA	TCTTATCATG						
GTAAAAAAG	TGACGTAAGA	TCAACACCAA	ACAGGTTTGA	GTAGTTACAT	AGAATAGTAC						
245	250	255	260	265	270	275	280	285	290	295	300
TCTGGATCTC	TAGCTTCGTG	TCAAGGACGG	TGACTGCGAGT	GAATAATAAA	ATGTGTGTTT						
AGACCTAGAG	ATCGAAGCAC	AGTTCCTGCC	ACTGACGTCA	CTTATTATTT	TACACACAAA						
305	310	315	320	325	330	335	340	345	350	355	360
GTCCGAAATA	CGCGTTTTGA	GATTTCTGTC	GCCGACTAAA	TTCATGTGCG	GCGATAGTGG						
CAGGCTTTAT	CGCGAAAAC	CTAAAGACAG	CGGCTGATTT	AAGTACAGCG	CGCTATCACC						
365	370	375	380	385	390	395	400	405	410	415	420
TGTTTATCGC	CGATAGAGAT	GGCGATATTG	GAAAAATCGA	TATTTGAAAA	TATGGCATAT						
ACAAATAGCG	GCTATCTCTA	CCGCTATAAC	CTTTTAGCT	ATAAACTTTT	ATACCGTATA						
425	430	435	440	445	450	455	460	465	470	475	480
TGAAAATGTC	GCCGATGTGA	GTTTCTGTGT	AACTGATATC	GCCATTTTTT	CAAAAGTGAT						
ACTTTTACAG	CGGCTACACT	CAAAGACACA	TTGACTATAG	GCCTAAAAAG	GTTTCACTA						
485	490	495	500	505	510	515	520	525	530	535	540
TTTTGGGCAT	ACGCGATATC	TGGCGATAGC	GCTTATATCG	TTTACGGGGG	ATGGCGATAG						
AAAACCCGTA	TGCGCTATAG	ACCGCTATCG	CGAATATAGC	AAATGCCCCC	TACCGCTATC						
545	550	555	560	565	570	575	580	585	590	595	600
ACGACTTTGG	TGACTTGGGC	GATTCTGTGT	GTCGCAAATA	TCGCAGTTTC	GATATAGGTG						
TGCTGAAACC	ACTGAACCCG	CTAAGACACA	CAGCGTTTAT	AGCGTCAAAG	CTATATCCAC						
605	610	615	620	625	630	635	640	645	650	655	660
ACAGACGATA	TGAGGCTATA	TCGCCGATAG	AGGCGACATC	AAGCTGGCAC	ATGGCCAATG						
TGTCTGCTAT	ACTCCGATAT	AGCGGCTATC	TCCGCTGTAG	TTCGACCGTG	TACCGGTTAG						
665	670	675	680	685	690	695	700	705	710	715	720
CATATCGATC	TATACATTGA	ATCAATATTG	GCCATTAGCC	ATATTATTCA	TTGGTTATAT						
GTATAGCTAG	ATATGTAAGT	TAGTTATAAC	CGGTAATCGG	TATAATAAGT	AACCAATATA						

FIG. 29A

RECTIFIED SHEET (RULE 91)

39/56

725 730 735 740 745 750 755 760 765 770 775 780
 * * * * *
 AGCATAAATC AATATTGGCT ATTGGCCATT GCATACGTTG TATCCATATC ATAATATGTA
 TCGTATTTAG TTATAACCGA TAACCGGTAA CGTATGCAAC ATAGGTATAG TATTATACAT
 785 790 795 800 805 810 815 820 825 830 835 840
 * * * * *
 CATTATATTT GGCTCATGTC CAACATTACC GCCATGTTGA CATTGATTAT TGACTIONGTA
 GTAAATATAA CCGAGTACAG GTTGTAATGG CCGTACAAC GTAACTAATA ACTGATCAAT
 845 850 855 860 865 870 875 880 885 890 895 900
 * * * * *
 TTAATAGTAA TCAATTACGG GGTCAATTAGT TCATAGCCCA TATATGGAGT TCCGCGTTAC
 AATTATCATT AGTTAATGCC CCAGTAATCA AGTATCGGGT ATATACCTCA AGGCGCAATG
 905 910 915 920 925 930 935 940 945 950 955 960
 * * * * *
 ATAACTTACG GTAAATGGCC CGCCTGGCTG ACCGCCCAAC GACCCCGGCC CATTGACGTC
 TATTGAATGC CATTIACCGG GCGGACCGAC TGGCGGGTTG CTGGGGGCGG GTAACTGCAG
 965 970 975 980 985 990 995 1000 1005 1010 1015 1020
 * * * * *
 AATAATGACG TATGTTCCCA TAGTAACGCC AATAGGGACT TTCCATTGAC GTCAATGGGT
 TTATTACTGC ATACAAGGGT ATCATTGCGG TTATCCCTGA AAGGTAAGTG CAGTTACCCA
 1025 1030 1035 1040 1045 1050 1055 1060 1065 1070 1075 1080
 * * * * *
 GGAGTATTTA CCGTAACTG CCCACTTGGC AGTACATCAA GTGTATCATA TGCCAAGTAC
 CCTCATAAAT GCCATTTGAC GGGTGAACCG TCATGTAGTT CACATAGTAT ACGGTTTCATG
 1085 1090 1095 1100 1105 1110 1115 1120 1125 1130 1135 1140
 * * * * *
 GCCCCCTATT GACGTCAATG ACGGTAAATG GCCCGCCTGG CATTATGCCC AGTACATGAC
 CCGGGGATAA CTGCAGTTAC TGCCATTTAC CCGGCGGACC GTAATACGGG TCATGTACTG
 1145 1150 1155 1160 1165 1170 1175 1180 1185 1190 1195 1200
 * * * * *
 CTTATGGGAC TTTCTACTT GGCAGTACAT CTACGTATTA GTCATCGCTA TTACCATGGT
 GAATACCCTG AAAGGATGAA CCGTCATGTA GATGCATAAT CAGTAGCGAT AATGGTACCA
 1205 1210 1215 1220 1225 1230 1235 1240 1245 1250 1255 1260
 * * * * *
 GATGCGGTTT TGGCAGTACA TCAATGGGCG TGGATAGCGG TTTGACTCAC GGGGATTTCC
 CTACGCCAAA ACCGTCAATG AGTTACCCGC ACCTATCGCC AAAGTGAAGT CCCCTAAAGC
 1265 1270 1275 1280 1285 1290 1295 1300 1305 1310 1315 1320
 * * * * *
 AAGTCTCCAC CCCATTGACG TCAATGGGAG TTTGTTTTGG CACCAAAATC AACGGGAGTT
 TTCAGAGGTG GGGTAACTGC AGTTACCCCTC AAACAAAACC GTGGTTTTAG TTGCCCTTAA
 1325 1330 1335 1340 1345 1350 1355 1360 1365 1370 1375 1380
 * * * * *
 TCCAAAATGT CGTAACAACT CCGCCCCATT GACGCAAAATG GGCGGTAGGC GTGTAACT
 AGGTTTTACA GCATTGTTGA GCGGGGTAA CTGCGTTTAC CCGCCATCCG CACATGTT
 1385 1390 1395 1400 1405 1410 1415 1420 1425 1430 1435 1440
 * * * * *
 GGAGGTCTAT ATAAGCAGAG CTCGTTTAGT GAACCGTCAG ATCGCCTGGA GACGCGTAA
 CCTCCAGATA TATTCGTCTC GAGCAAAATCA CTTGGCAGTC TAGCGGACCT CTGCGGTTAA

FIG. 29B

RECTIFIED SHEET (RULE 91)

40/56

1445 1450 *	1455 1460 *	1465 1470 *	1475 1480 *	1485 1490 *	1495 1500 *
ACGCTGTTTT	GACCTCCATA	GAAGACACCG	GGACCGATCC	AGCCTCCGCG	GCCGGGAACG
TGCGACAAAA	CTGGAGGTAT	CTTCTGTGGC	CCTGGCTAGG	TCGGAGGCGC	CGGCCCTTGC
1505 1510 *	1515 1520 *	1525 1530 *	1535 1540 *	1545 1550 *	1555 1560 *
GTGCATTGGA	ACGCGGATTC	CCCGTGCCAA	GAGTGACGTA	AGTACCGCCT	ATAGAGTCTA
CACGTAACCT	TGCGCCTAAG	GGGCACGGTT	CTCACTGCAT	TCATGGCGGA	TATCTCAGAT
1565 1570 *	1575 1580 *	1585 1590 *	1595 1600 *	1605 1610 *	1615 1620 *
TAGGCCCAAC	CCCTTGGCTT	CTTATGCATG	CTATACTGTT	TTTGGCTTGG	GGTCTATACA
ATCCGGGTGG	GGGAACCGAA	GAATACGTAC	GATATGACAA	AAACCGAACC	CCAGATATGT
1625 1630 *	1635 1640 *	1645 1650 *	1655 1660 *	1665 1670 *	1675 1680 *
CCCCCGCTTC	CTCATGTTAT	AGGTGATGGT	ATAGCTTAGC	CTATAGGTGT	GGGTTATTGA
GGGGGCGAAG	GAGTACAATA	TCCACTACCA	TATCGAATCG	GATATCCACA	CCCAATAACT
1685 1690 *	1695 1700 *	1705 1710 *	1715 1720 *	1725 1730 *	1735 1740 *
CCATTATTGA	CCACTCCCCT	ATTGGTGACG	ATACTTTCCA	TTACTAATCC	ATAACATGGC
GGTAATAACT	GGTGAGGGGA	TAACCACTGC	TATGAAAGGT	AATGATTAGG	TATTGTACCG
1745 1750 *	1755 1760 *	1765 1770 *	1775 1780 *	1785 1790 *	1795 1800 *
TCTTTGCCAC	AACTCTCTTT	ATTGGCTATA	TGCCAATACA	CTGTCCTTCA	GAGACTGACA
AGAAACGGTG	TTGAGAGAAA	TAACCGATAT	ACGGTTATGT	GACAGGAAGT	CTCTGACTGT
1805 1810 *	1815 1820 *	1825 1830 *	1835 1840 *	1845 1850 *	1855 1860 *
CGGACTCTGT	ATTTTACAG	GATGGGGTCT	CATTATTAT	TTACAAATTC	ACATATACAA
GCCTGAGACA	TAAAAATGTC	CTACCCCGAG	GTAAATAATA	AATGTTTAAAG	TGTATATGTT
1865 1870 *	1875 1880 *	1885 1890 *	1895 1900 *	1905 1910 *	1915 1920 *
CACCACCGTC	CCCAGTGCCC	GCAGTTTTTA	TTAAACATAA	CGTGGGATCT	CCACCGGAAT
GTGGTGGCAG	GGGTCACGGG	CGTCAAAAAT	AATTTGTATT	GCACCCTAGA	GGTGCGCTTA
1925 1930 *	1935 1940 *	1945 1950 *	1955 1960 *	1965 1970 *	1975 1980 *
CTCGGGTACG	TGTTCCGGAC	ATGGGCTCTT	CTCCGGTAGC	GGCGGAGCTT	CTACATCCGA
GAGCCCATGC	ACAAGGCCTG	TACCCGAGAA	GAGGCCATCG	CCGCCTCGAA	GATGTAGGCT
1985 1990 *	1995 2000 *	2005 2010 *	2015 2020 *	2025 2030 *	2035 2040 *
GCCCTGCTCC	CATGCCTCCA	GCGACTCATG	GTCGCTCGGC	AGCTCCTTGC	TCCTAACAGT
CGGGACGAGG	GTACGGAGGT	CGCTGAGTAC	CAGCGAGCCG	TCGAGGAACG	AGGATTGTCA
2045 2050 *	2055 2060 *	2065 2070 *	2075 2080 *	2085 2090 *	2095 2100 *
GGAGGCCACA	CTTAGGCACA	GCACGATGCC	CACCACCACC	AGTGTGCCGC	ACAAGGCCGT
CCTCCGGTCT	GAATCCGTGT	CGTGCTACGG	GTGGTGGTGG	TCACACGGCG	TGTTCCGGCA
2105 2110 *	2115 2120 *	2125 2130 *	2135 2140 *	2145 2150 *	2155 2160 *
GGCGGTAGGG	TATGTGTCTG	AAAATGAGCT	CGGGGAGCGG	GCTTGACCCG	CTGACGCATT
CCGCCATCCC	ATACACAGAC	TTTACTCGA	GCCCCTCGCC	CGAACGTGGC	GACTGCGTAA

FIG. 29C

RECTIFIED SHEET (RULE 91)

41/56

2165	2170	2175	2180	2185	2190	2195	2200	2205	2210	2215	2220
TGGAAGACTT	AAGGCAGCGG	CAGAAGAAGA	TGCAGGCAGC	TGAGTTGTTG	TGTTCTGATA	ACCTTCTGAA	TTCCGTGCGC	GTCTTCTTCT	ACGTCCGTCG	ACTCAACAAC	ACAAGACTAT
2225	2230	2235	2240	2245	2250	2255	2260	2265	2270	2275	2280
AGAGTCAGAG	GTAAC TCCCG	TTGCGGTGCT	GTTAACGGTG	GAGGGCAGTG	TAGTCTGAGC	TCTCAGTCTC	CATTGAGGGC	AACGCCACGA	CAATTGCCAC	CTCCCGTCAC	ATCAGACTCG
2285	2290	2295	2300	2305	2310	2315	2320	2325	2330	2335	2340
AGTACTCGTT	GCTGCCGCGC	GCGCCACCAG	ACATAATAGC	TGACAGACTA	ACAGACTGTT	TCATGAGCAA	CGACGGCGCG	CGCGGTGGTG	TGTATTATCG	ACTGTCTGAT	TGTCTGACAA
2345	2350	2355	2360	2365	2370	2375	2380	2385	2390	2395	2400
CCTTTCATG	GGTCTTTTCT	GCAGTCACCG	TCCTTGACAC	GAAGCTTGGG	CTGCAGGTCC	GGAAAGGTAC	CCAGAAAAGA	CGTCAGTGCG	AGGAACTGTG	CTTCGAACCC	GACGTCCAGC
2405	2410	2415	2420	2425	2430	2435	2440	2445	2450	2455	2460
ATCGACTCTA	GAGGATCGAT	CCCCGGGCGA	GCTCGAATTC	GCCGCCACCA	TGGAATGGAG	TAGCTGAGAT	CTCCTAGCTA	GGGGCCCGCT	CGAGCTTAAG	CGGCGGTGGT	ACCTTACCTC
2465	2470	2475	2480	2485	2490	2495	2500	2505	2510	2515	2520
CTGGGTCTTT	CTCTTCTTCC	TGTCAGTAAC	TACAGGTGTC	CACTCCCAGG	TTCAGCTGGT	GACCCAGAAA	GAGAAGAAGG	ACAGTCATTG	ATGTCCACAG	GTGAGGGTCC	AAGTCGACCA
2525	2530	2535	2540	2545	2550	2555	2560	2565	2570	2575	2580
TCAGTCCGGG	GCTGAGGTGA	AGAAGCCTGG	GGCCTCAGTG	AAGGTTTCTT	GTCAGGCTTC	AGTCAGGCCC	CGACTCCACT	TCTTCGGACC	CCGGAGTCAC	TTCCAAAGAA	CAGTCCGAAG
2585	2590	2595	2600	2605	2610	2615	2620	2625	2630	2635	2640
TGGATACAGA	TTCAGTAACT	TTGTTATTCA	TTGGGTGCGC	CAGGCCCCCG	GACAGAGGTT	ACCTATGTCT	AAGTCATTGA	AACAATAAGT	AACCCACGCG	GTCCGGGGGC	CTGTCTCCAA
2645	2650	2655	2660	2665	2670	2675	2680	2685	2690	2695	2700
TGAGTGGATG	GGATGGATCA	ATCCTTACAA	CGGAAACAAA	GAATTTTCAG	CGAAGTTCCA	ACTCACCTAC	CCTACCTAGT	TAGGAATGTT	GCCTTTGTTT	CTTAAAAGTC	GCTTCAAGGT
2705	2710	2715	2720	2725	2730	2735	2740	2745	2750	2755	2760
GGACAGAGTC	ACCTTTACCG	CGGACACATC	CGCGAACACA	GCCTACATGG	AGTTGAGGAG	CCTGTCTCAG	TGGAAATGGC	GCCTGTGTAG	GCGCTTGTGT	CGGATGTACC	TCAACTCCTC
2765	2770	2775	2780	2785	2790	2795	2800	2805	2810	2815	2820
CCTCAGGTCT	GCAGACACGG	CTGTTTATTA	TTGTGCGAGA	GTGGGGCCAT	ATAGTTGGGA	GGAGTCCAGA	CGTCTGTGCC	GACAAATAAT	AACACGCTCT	CACCCCGGTA	TATCAACCCT
2825	2830	2835	2840	2845	2850	2855	2860	2865	2870	2875	2880
TGATTCTCCC	CAGGACAATT	ATTATATGGA	CGTCTGGGGC	AAAGGAACCA	CGGTCAATCGT	ACTAAGAGGG	GTCCTGT TAA	TAATATACCT	GCAGACCCCG	TTTCCTTG GT	CCCAGTAGCA

FIG. 29D

RECTIFIED SHEET (RULE 91)

42/56

2885	2890	2895	2900	2905	2910	2915	2920	2925	2930	2935	2940
GAGCTCAGCT	TCCACCAAGG	GCCCCATCGGT	CTTCCCCCTG	GCACCCTCCT	CCAAGAGCAC	CTCGAGTCGA	AGGTGGTTCC	CGGGTAGCCA	GAAGGGGGAC	CGTGGGAGGA	GGTTCTCTGT
2945	2950	2955	2960	2965	2970	2975	2980	2985	2990	2995	3000
CTCTGGGGGG	ACAGCGGGCC	TGGGCTGCCT	GGTCAAGGAC	TACTTCCCCG	AACCGGTGAC	GAGACCCCGG	TGTCGCCGGG	ACCCGACGGA	CCAGTTCCTG	ATGAAGGGGC	TTGGCCACTG
3005	3010	3015	3020	3025	3030	3035	3040	3045	3050	3055	3060
GGTGTCTGTG	AACTCAGGCG	CCCTGACCAG	CGGCGTGCAC	ACCTTCCCCG	CTGTCTTACA	CCACAGCACC	TTGAGTCCGC	GGGACTGGTC	GCCGCACGTG	TGGAAGGGCC	GACAGGATGT
3065	3070	3075	3080	3085	3090	3095	3100	3105	3110	3115	3120
GTCCTCAGGA	CTCTACTCCC	TCAGCAGCGT	GGTGACCGTG	CCCTCCAGCA	GCTTGGGCAC	CAGGAGTCCT	GAGATGAGGG	AGTCGTCGCA	CCACTGGCAC	GGGAGGTCGT	CGAACCCGTG
3125	3130	3135	3140	3145	3150	3155	3160	3165	3170	3175	3180
CCAGACCTAC	ATCTGCAACG	TGAATCACAA	GCCCAGCAAC	ACCAAGGTGG	ACAAGAAAGT	GGTCTGGATG	TAGACGTTGC	ACTTAGTGTT	CGGGTCGTTG	TGGTTCCACC	TGTTCTTTCA
3185	3190	3195	3200	3205	3210	3215	3220	3225	3230	3235	3240
TGGTGAGAGG	CCAGCACAGG	GAGGGAGGGT	GTCTGTCTGA	AGCCAGGCTC	AGCGCTCCTG	ACCACTCTCC	GGTCGTGTCC	CTCCCTCCCA	CAGACGACCT	TCGGTCCGAG	TCGCGAGGAC
3245	3250	3255	3260	3265	3270	3275	3280	3285	3290	3295	3300
CCTGGACGCA	TCCCGGCTAT	GCAGCCCCAG	TCCAGGGCAG	CAAGGCAGGC	CCCCTCTGCC	GGACCTGCGT	AGGGCCGATA	CGTCGGGGTC	AGGTCCCGTC	GTTCCGTCCG	GGGCAGACGG
3305	3310	3315	3320	3325	3330	3335	3340	3345	3350	3355	3360
TCTTCACCCG	GAGGCCTCTG	CCCGCCCCAC	TCATGCTCAG	GGAGAGGGTC	TTCTGGCTTT	AGAAGTGGGC	CTCCGGAGAC	GGGCGGGGTG	AGTACGAGTC	CCTCTCCCAG	AAGACCGAAA
3365	3370	3375	3380	3385	3390	3395	3400	3405	3410	3415	3420
TTCCCCAGGC	TCTGGGCAGG	CACAGGCTAG	GTGCCCCCTAA	CCCAGGCCCT	GCACACAAAG	AAGGGGTCCG	AGACCCGTCC	GTGTCCGATC	CACGGGGATT	GGGTCCGGGA	CGTGTGTTTC
3425	3430	3435	3440	3445	3450	3455	3460	3465	3470	3475	3480
GGGCAGGTGC	TGGGCTCAGA	CCTGCCAAGA	GCCATATCCG	GGAGGACCCT	GCCCCTGACC	CCCGTCCACG	ACCCGAGTCT	GGACGGTTCT	CGGTATAGGC	CCTCCTGGGA	CGGGGACTGG
3485	3490	3495	3500	3505	3510	3515	3520	3525	3530	3535	3540
TAAGCCCACC	CCAAAGGCCA	AACTCTCCAC	TCCCTCAGCT	CGGACACCTT	CTCTCCTCCC	ATTGCGGTGG	GGTTTCCGGT	TTGAGAGGTG	AGGGAGTCGA	GCCTGTGGAA	GAGAGGAGGG
3545	3550	3555	3560	3565	3570	3575	3580	3585	3590	3595	3600
AGATTTCGAGT	AACTCCCAAT	CTTCTCTCTG	CAGAGCCCAA	ATCTTGTTGAC	AAAACCTACA	TCTAAGCTCA	TTGAGGGTTA	GAAGAGAGAC	GTCTCGGGTT	TAGAACACTG	TTTTGAGTGT

FIG. 29E

RECTIFIED SHEET (RULE 91)

43/56

3605 3610	3615 3620	3625 3630	3635 3640	3645 3650	3655 3660
CATGCCCACC	GTGCCCAGGT	AAGCCAGCCC	AGGCCTCGCC	CTCCAGCTCA	AGGCGGGACA
GTACGGGTGG	CACGGGTCCA	TTCGGTCGGG	TCCGGAGCGG	GAGGTCGAGT	TCCGCCCTGT
3665 3670	3675 3680	3685 3690	3695 3700	3705 3710	3715 3720
GGTGCCCTAG	AGTAGCCTGC	ATCCAGGGAC	AGGCCCCAGC	CGGGTGCTGA	CACGTCCACC
CCACGGGATC	TCATCGGACG	TAGGTCCCTG	TCCGGGGTCG	GCCCACGACT	GTGCAGGTGG
3725 3730	3735 3740	3745 3750	3755 3760	3765 3770	3775 3780
TCCATCTCTC	CCTCAGCACC	TGAGGCCGCG	GGAGGACCAT	CAGTCTTCCT	CTTCCCCCCA
AGGTAGAGAG	GGAGTCGTGG	ACTCCGGCGC	CCTCCTGGTA	GTGAGAAGGA	GAAGGGGGGT
3785 3790	3795 3800	3805 3810	3815 3820	3825 3830	3835 3840
AAACCCAAGG	ACACCCTCAT	GATCTCCCGG	ACCCCTGAGG	TCACATGCGT	GGTGGTGGAC
TTTGGGTTCC	TGTGGGAGTA	CTAGAGGGCC	TGGGGACTCC	AGTGTACGCA	CCACCACCTG
3845 3850	3855 3860	3865 3870	3875 3880	3885 3890	3895 3900
GTGAGCCACG	AAGACCCTGA	GGTCAAGTTC	AACTGGTACG	TGGACGGCGT	GGAGGTGCAT
CACTCGGTGC	TTCTGGGACT	CCAGTTCAAG	TTGACCATGC	ACCTGCCGCA	CCTCCACGTA
3905 3910	3915 3920	3925 3930	3935 3940	3945 3950	3955 3960
AATGCCAAGA	CAAAGCCGCG	GGAGGAGCAG	TACAACAGCA	CGTACCGTGT	GGTCAGCGTC
TTACGGTTCT	GTTTCGGCGC	CCTCCTCGTC	ATGTTGTCTG	GCATGGCACA	CCAGTCGCAG
3965 3970	3975 3980	3985 3990	3995 4000	4005 4010	4015 4020
CTCACCGTCC	TGCACCAGGA	CTGGCTGAAT	GGCAAGGAGT	ACAAGTGCAA	GGTCTCCAAC
GAGTGGCAGG	ACGTGGTCCT	GACCGACTTA	CCGTTCTCTA	TGTTACGTT	CCAGAGGTTG
4025 4030	4035 4040	4045 4050	4055 4060	4065 4070	4075 4080
AAAGCCCTCC	CAGCCCCCAT	CGAGAAAACC	ATCTCCAAAG	CCAAAGGTGG	GACCCGTGGG
TTTCGGGAGG	GTGCGGGGTA	GCTCTTTTGG	TAGAGGTTTC	GGTTTCACC	CTGGGCACCC
4085 4090	4095 4100	4105 4110	4115 4120	4125 4130	4135 4140
GTGCGAGGGC	CACATGGACA	GAGGCCGGCT	CGGCCACCC	TCTGCCCTGA	GAGTGACCGC
CACGCTCCCG	GTGTACCTGT	CTCCGGCCGA	GCCGGGTGGG	AGACGGGACT	CTCACTGGCG
4145 4150	4155 4160	4165 4170	4175 4180	4185 4190	4195 4200
TGTACCAACC	TCTGTCCCTA	CAGGGCAGCC	CCGAGAACCA	CAGGTGTACA	CCCTGCCCCC
ACATGGTTGG	AGACAGGGAT	GTCCCGTCGG	GGCTCTTGGT	GTCCACATGT	GGGACGGGGG
4205 4210	4215 4220	4225 4230	4235 4240	4245 4250	4255 4260
ATCCCGGGAT	GAGCTGACCA	AGAACCAGGT	CAGCCTGACC	TGCCTGGTCA	AAGGCTTCTA
TAGGGCCCTA	CTCGACTGGT	TCTTGGTCCA	GTCGGACTGG	ACGGACCAGT	TTCCGAAGAT
4265 4270	4275 4280	4285 4290	4295 4300	4305 4310	4315 4320
TCCCAGCGAC	ATCGCCGTGG	AGTGGGAGAG	CAATGGGCAG	CCGGAGAACA	ACTACAAGAC
AGGGTCGCTG	TAGCGGCACC	TCACCCTCTC	GTTACCCGTC	GGCCTCTTGT	TGATGTTCTG

FIG. 29F

RECTIFIED SHEET (RULE 91)

44/56

4325	4330	4335	4340	4345	4350	4355	4360	4365	4370	4375	4380
CACGCCTCCC	GTGCTGGACT	CCGACGGCTC	CTTCTTCCTC	TACAGCAAGC	TCACCGTGGA						
GTGCGGAGGG	CACGACCTGA	GGCTGCCGAG	GAAGAAGGAG	ATGTCGTTCC	AGTGGCACCT						
4385	4390	4395	4400	4405	4410	4415	5520	4425	4430	4435	4440
CAAGAGCAGG	TGGCAGCAGG	GGAACGTCTT	CTCATGCTCC	GTGATGCATG	AGGCTCTGCA						
GTTCTCGTCC	ACCGTCGTCC	CCTTGCAGAA	GAGTACGAGG	CACTACGTAC	TCCGAGACGT						
4445	4450	4455	4460	4465	4470	4475	4480	4485	4490	4495	4500
CAACCACTAC	ACGCAGAAGA	GCCTCTCCCT	GTCTCCGGGT	AAATGAGTGC	GACGGCCGGC						
GTTGGTGATG	TGCGTCTTCT	CGGAGAGGGA	CAGAGGCCCA	TTTACTCAGC	CTGCCGGCCG						
4505	4510	4515	4520	4525	4530	4535	4540	4545	4550	4555	4560
AAGCCCCCGC	TCCCCGGGCT	CTCGCGGTCC	CACGAGGATG	CTTGGCACGT	ACCCCTGTGA						
TTCGGGGGCG	AGGGGCCCGA	GAGCGCCAGC	GTGCTCCTAC	GAACCGTGCA	TGGGGGACAT						
4565	4570	4575	4580	4585	4590	4595	4600	4605	4610	4615	4620
CATACTTCCC	GGGCGCCCAG	CATGGAATA	AAGCACCCAG	CGCTGCCCTG	GGCCCTGCGG						
GTATGAAGGG	CCGCGGGGTC	GTACCTTTAT	TTCGTGGGTC	GCGACGGGAC	CCGGGGACGC						
4625	4630	4635	4640	4645	4650	4655	4660	4665	4670	4675	4680
AGACTGTGAT	GGTTCCTTCC	ACGGGTCAGG	CCGAGTCTGA	GGCCTGAGTG	GCATGAGGGA						
TCTGACACTA	CCAAGAAAGG	TGCCCAGTCC	GGCTCAGACT	CCGGACTCAC	CGTACTCCCT						
4685	4690	4695	4700	4705	4710	4715	4720	4725	4730	4735	4740
GGCAGAGCGG	GTCCCACTGT	CCCCACACTG	GCCCAGGCTG	TGCAGGTGTG	CCTGGGCCCGC						
CCGTCTCGCC	CAGGGTGACA	GGGGTGTGAC	CGGGTCCGAC	ACGTCCACAC	GGACCCGCGC						
4745	4750	4755	4760	4765	4770	4775	4780	4785	4790	4795	4800
CTAGGGTGGG	GCTCAGCCAG	GGGCTGCCCT	CGGCAGGGTG	GGGGATTTCG	CAGCGTTGCC						
GATCCACACC	CGAGTCGGTC	CCCGACGGGA	GCCGTCCCAC	CCCCTAAACG	GTCCGAACGG						
4805	4810	4815	5820	4825	4830	4835	4840	4845	4850	4855	4860
CTCCCTCCAG	CAGCACCTGC	CCTGGGCTGG	GCCACGGGAA	GCCCTAGGAG	CCCCTGGGGA						
GAGGGAGGTC	GTCGTGGACG	GGACCCGACC	CGGTGCCCTT	CGGGATCCTC	GGGGACCCCT						
4865	4870	4875	4880	4885	4890	4895	4900	4905	4910	4915	4920
CAGACACACA	GCCCCTGCCT	CTGTAGGAGA	CTGTCCTGTT	CTGTGAGCGC	CCTGTCTCTT						
GTCTGTGTGT	CGGGGACGGA	GACATCCTCT	GACAGGACAA	GACACTCGCG	GGACAGGA						
4925	4930	4935	4940	4945	4950	4955	4960	4965	4970	4975	4980
GACCTCCATG	CCCACTCGGG	GGCATGCCTA	GTCCATGTGC	GTAGGGACAG	GCCCTCTCTT						
CTGGAGGTAC	GGGTGAGCCC	CCGTACGGAT	CAGGTACACG	CATCCCTGTC	CGGGAG						
4985	4990	4995	5000	5005	5010	5015	5020	5025	5030	5035	
ACCCATCTAC	CCCCACGGCA	CTAACCCCTG	GCTGTCTCTG	CCAGCCTCGC	ACCCGTA						
TGGGTAGATG	GGGGTGCCGT	GATTGGGGAC	CGACAGGACG	GGTCGGAGCG	TGGGCTA						

FIG. 29G

RECTIFIED SHEET (RULE 91)

45/56

5045	5050	5055	5060	5065	5070	5075	5080	5085	5090	5095	5100
GGACACAACC	GA	CTCCGGGG	ACATGCACTC	TCGGGGCCCTG	TGGAGGGACT	GGTGCAGATG					
CCTGTGTTGG	CTGAGGCCCC	TGTACGTGAG	AGCCCCGGGAC	ACCTCCCTGA	CCACGTCTAC						
5105	5110	5115	5120	5125	5130	5135	5140	5145	5150	5155	5160
CCCACACACA	CACTCAGTCC	AGACCCGTTC	AACAAAACCC	CCGCACTGAG	GTTGGCCGGC						
GGGTGTGTGT	GTGAGTCAGG	TCTGGGCAAG	TTGTTTTGGG	GGCGTGA	CTC						
5165	5170	5175	5180	5185	5190	5195	5200	5205	5210	5215	5220
CACACGGCCA	CCACACACAC	ACGTGCACGC	CTCACACACG	GAGCCTCACC	CGGGCGAACT						
GTGTGCCGGT	GGTGTGTGTG	TGCACGTGCG	GAGTGTGTGC	CTCGGAGTGG	GCCCCGTGA						
5225	5230	5235	5240	5245	5250	5255	5260	5265	5270	5275	5280
GCACAGCACC	CAGACCAGAG	CAAGGTCTCT	GCACACGTGA	ACACTCCTCG	GACACAGGCC						
CGTGTCTGTG	GTCTGGTCTC	GTTCCAGGAG	CGTGTGCACT	TGTGAGGAGC	CTGTGTCCCG						
5285	5290	5295	5300	5305	5310	5315	5320	5325	5330	5335	5340
CCCACGAGCC	CCACGCGGCA	CCTCAAGGCC	CACGAGCCTC	TCGGCAGCTT	CTCCACATGC						
GGGTGCTCGG	GGTGCGCCGT	GGAGTTCGGG	GTGCTCGGAG	AGCCGTCGAA	GAGGTGTACC						
5345	5350	5355	5360	5365	5370	5375	5380	5385	5390	5395	5400
TGACCTGCTC	AGACAAAACCC	AGCCCTCCTC	TCACAAGGGT	CCCCCTGCAG	CCGCCACACA						
ACTGGACGAG	TCTGTTTGGG	TCGGGAGGAG	AGTGTTC	CGGGGACGTC	GGCGGTGTGT						
5405	5410	5415	5420	5425	5430	5435	5440	5445	5450	5455	5460
CACACAGGGG	ATCACACACC	ACGTACACGT	CCTGGCCCTG	GCCCACTTCC	CAGTGCCGCG						
GTGTGTCCCC	TAGTGTGTGG	TGCAGTGCAG	GGACCGGGAC	CGGGTGAAGG	GTCACGGCGG						
5465	5470	5475	5480	5485	5490	5495	5500	5505	5510	5515	5520
CTTCCCTGCA	GGGCGGATCA	TAATCAGCCA	TACCACATTT	GTAGAGGTTT	TACTTGCTTT						
GAAGGGACGT	CCCGCCTAGT	ATTAGTCGGT	ATGGTGTA	CATCTCCAAA	ATGAACGAAA						
5525	5530	5535	5540	5545	5550	5555	5560	5565	5570	5575	5580
AAAAAACCTC	CCACACCTCC	CCCTGAACCT	GAAACATAAA	ATGAATGCAA	TTGTTGTTGT						
TTTTTTGGAG	GGTGTGGAGG	GGGACTTGGA	CTTTGTATTT	TACTTACGTT	AACAACAACA						
5585	5590	5595	5600	5605	5610	5615	5620	5625	5630	5635	5640
TAACTTGTTT	ATTGCAGCTT	ATAATGGTTA	CAAATAAAGC	AATAGCATCA	CAAATTTTAC						
ATTGAACAAA	TAACGTCGAA	TATTACCAAT	GTTTATTTTCG	TTATCGTAGT	GTTTAAAGTG						
5645	5650	5655	5660	5665	5670	5675	5680	5685	5690	5695	5700
AAATAAAGCA	TTTTTTTCAC	TGCATTCTAG	TTGTGGTTTG	TCCAAACTCA	TCAATGTATC						
TTTATTTTCG	AAAAAAAGTG	ACGTAAGATC	AACACCAAAC	AGGTTTGAGT	AGTTACATAG						
5750	5710	5715	5720	5725	5730	5735	5740	5745	5750	5755	5760
TTATCATGTC	TGAGATCCTC	TACGCCGGAC	GCATCGTGCC	CGGCATCACC	GGCGCCACAG						
AATAGTACAG	ACTCTAGGAG	ATGCGGCCTG	CGTAGCACCG	GCCGTAGTGG	CCGCGGTGTC						

FIG. 29H

RECTIFIED SHEET (RULE 90)

46/56

5765	5770	5775	5780	5785	5790	5795	5800	5805	5810	5815	5820
GTGCGGTTGC	TGGCGCCTAT	ATCGCCGACA	TCACCGATGG	GGAAGATCGG	GCTCGCCACT						
CACGCCAACG	ACCGCGGATA	TAGCGGCTGT	AGTGGCTACC	CCTTCTAGCC	CGAGCGGTGA						
5825	5830	5835	5840	5845	5850	5855	5860	5865	5870	5875	5880
TCGGGCTCAT	GAGCGCTTGT	TTCGGCGTGG	GTATGGTGGC	AGGCCCGTGG	CCGGGGGACT						
AGCCCGAGTA	CTCGCGAACA	AAGCCGCACC	CATACCACCG	TCCGGGCACC	GGCCCCCTGA						
5885	5890	5895	5900	5905	5910	5915	5920	5925	5930	5935	5940
GTTGGGCGCC	ATCTCCTTGC	ATGCACCAAT	CCTTGCGGCG	GCGGTGCTCA	ACGGCCTCAA						
CAACCCGCGG	TAGAGGAACG	TACGTGGTAA	GGAACGCCGC	CGCCACGAGT	TGCCGGAGTT						
5945	5950	5955	5960	5965	5970	5975	5980	5985	5990	5990	6000
CCTACTACTG	GGCTGCTTCC	TAATGCAGGA	GTGCGATAAG	GGAGAGCGTC	GACCTCGGGC						
GGATGATGAC	CCGACGAAGG	ATTACGTCTT	CAGCGTATTG	CCTCTCGCAG	CTGGAGCCCC						
6005	6010	6015	6020	6025	6030	6035	6040	6045	6050	6055	6060
CGCGTTGCTG	GCGTTTTTCC	ATAGGCTCCG	CCCCCCTGAC	GAGCATCACA	AAAATCGACG						
GCGCAACGAC	CGCAAAAAGG	TATCCGAGGC	GGGGGGACTG	CTCGTAGTGT	TTTATAGTGC						
6065	6070	6075	6080	6085	6090	6095	6100	6105	6110	6115	6120
CTCAAGTCAG	AGGTGGCGAA	ACCCGACAGG	ACTATAAAGA	TACCAGGCGT	TTCCCCCTGG						
GAGTTCAGTC	TCCACCGCTT	TGGGCTGTCC	TGATATTTCT	ATGGTCCGCA	AAGGGGGACC						
6125	6130	6135	6140	6145	6150	6155	6160	6165	6170	6175	6180
AAGCTCCCTC	GTGCGCTCTC	CTGTTCCGAC	CCTGCCGCTT	ACCGGATACC	TGTCCGCCTT						
TTCGAGGGAG	CACGCGAGAG	GACAAGGCTG	GGACGGCGAA	TGGCCTATGG	ACAGGCGGAA						
6185	6190	6195	6200	6205	6210	6215	6220	6225	6230	6235	6240
TCTCCCTTCG	GGAAGCGTGG	CGCTTTCTCA	ATGCTCACGC	TGTAGGTATC	TCAGTTCGGT						
AGAGGGAAGC	CCTTCGCACC	GCGAAAGAGT	TACGAGTGCG	ACATCCATAG	AGTCAAGCCA						
6245	6250	6255	6260	6265	6270	6275	6280	6285	6290	6295	6300
GTAGGTGCGT	CGCTCCAAGC	TGGGCTGTGT	GCACGAACCC	CCCGTTTCAGC	CCGACCGCTG						
CATCCAGCAA	GCGAGGTTCC	ACCCGACACA	CGTGCTTGGG	GGGCAAGTCG	GGCTGGCGAC						
6305	6310	6315	6320	6325	6330	6335	6340	6345	6350	6355	6360
CGCCTTATCC	GGTAACTATC	GTCTTGAGTC	CAACCCGGTA	AGACACGACT	TATCGCCACT						
GCGGAATAGG	CCATTGATAG	CAGAACTCAG	GTTGGGCCAT	TCTGTGCTGA	ATAGCGGTGA						
6365	6370	6375	6380	6385	6390	6395	6400	6405	6410	6415	6420
GGCAGCAGCC	ACTGGTAACA	GGATTAGCAG	AGCGAGGTAT	GTAGGCGGTG	CTACAGAGTT						
CCGTGCTCGG	TGACCATTGT	CCTAATCGTC	TCGCTCCATA	CATCCGCCAC	GATGTCTCAA						
6425	6430	6435	6440	6445	6450	6455	6460	6465	6470	6475	6480
CTTGAAGTGG	TGGCCTAACT	ACGGCTACAC	TAGAAGGACA	GTATTTGGTA	TCTGCGCTCT						
GAACCTTACC	ACCGGATTGA	TGCCGATGTG	ATCTTCCTGT	CATAAACCAT	AGACGCCGAGA						

FIG. 291

RECTIFIED SHEET (RULE 91)

47/56

```

6485 6490 6495 6500 6505 6510 6515 6520 6525 6530 6535 6540
*      *      *      *      *      *      *      *
GCTGAAGCCA GTTACCTTCG GAAAAAGAGT TGGTAGCTCT TGATCCGGCA AACAAACCAC
CGACTTCGGT CAATGGAAGC CTTTTTCTCA ACCATCGAGA ACTAGGCCGT TTGTTTGGTG

6545 6550 6555 6560 6565 6570 6575 6580 6585 6590 6595 6600
*      *      *      *      *      *      *      *
CGCTGGTAGC GGTGGTTTTT TTGTTTGCAA GCAGCAGATT ACGCGCAGAA AAAAAGGATC
GCGACCATCG CCACCAAAAA AACAAACGTT CGTCGTCTAA TCGCGGTCTT TTTTTCCTAG

6605 6610 6615 6620 6625 6630 6635 6640 6645 6650 6655 6660
*      *      *      *      *      *      *      *
TCAAGAAGAT CCTTGATCT TTTCTACGGG GTCTGACGCT CAGTGGAAACG AAAACTCACC
AGTTCTTCTA GGAAACTAGA AAAGATGCCC CAGACTGCGA GTCACCTTGC TTTTGAGTGC

6665 6670 6675 6680 6685 6690 6695 6700 6705 6710 6715 6720
*      *      *      *      *      *      *      *
TTAAGGGATT TTGGTCATGA GATTATCAAA AAGGATCTTC ACCTAGATCC TTTTAAATTA
AATTCCCTAA AACCAGTACT CTAATAGTTT TTCCTAGAAG TGGATCTAGG AAAATTTAAT

6725 6730 6735 6740 6745 6750 6755 6760 6765 6770 6775 6780
*      *      *      *      *      *      *      *
AAAATGAAGT TTTAAATCAA TCTAAAGTAT ATATGAGTAA ACTTGGTCTG ACAGTTACCA
TTTTACTTCA AAATTTAGTT AGATTTTCATA TATACTCATT TGAACCAGAC TGTCAATGGT

6785 6790 6795 6800 6805 6810 6815 6820 6825 6830 6835 6840
*      *      *      *      *      *      *      *
ATGCTTAATC AGTGAGGCAC CTATCTCAGC GATCTGTCTA TTTCGTTTCAT CCATAGTTGC
TACGAATTAG TCACTCCGTG GATAGAGTCG CTAGACAGAT AAAGCAAGTA GGTATCAACG

6845 6850 6855 6860 6865 6870 6875 6880 6885 6890 6895 6900
*      *      *      *      *      *      *      *
CTGACTCCCC GTCGTGTAGA TAACTACGAT ACGGGAGGGC TTACCATCTG GCCCCAGTGC
GACTGAGGGG CAGCACATCT ATTGATGCTA TGCCCTCCCG AATGGTAGAC CGGGGTCACC

6905 6910 6915 6920 6925 6930 6935 6940 6945 6950 6955 6960
*      *      *      *      *      *      *      *
TGCAATGATA CCGCGAGACC CACGCTCACC GGCTCCAGAT TTATCAGCAA TAAACCAGCC
ACGTTACTAT GGCGCTCTGG GTGCGAGTGG CCGAGGTCTA AATAGTCGTT ATTTGGTCGG

6965 6970 6975 6980 6985 6990 6995 7000 7005 7010 7015 7020
*      *      *      *      *      *      *      *
AGCCGGAAGG GCCGAGCGCA GAAGTGGTCC TGCAACTTTA TCCGCCTCCA TCCAGTCTAT
TCGGCCTTCC CGGCTCGCGT CTTACCAGG AC GTTGAAT AGCGGAGGT AGGTCAAGATA

7025 7030 7035 7040 7045 7050 7055 7060 7065 7070 7075 7080
*      *      *      *      *      *      *      *
TAATTGTTGC CGGGAAGCTA GAGTAAGTAG TTCGCCAGTT AATAGTTTGC GCAACGTTGT
ATTAACAACG GCCCTTCGAT CTCATTTCAT AAGCGGTCAA TTATCAAACG CGTTGCAACA

7085 7090 7095 7100 7105 7110 7115 7120 7125 7130 7135 7140
*      *      *      *      *      *      *      *
TGCCATTGCT ACAGGCATCG TGGTGTACG CTCGTCGTTT GGTATGGCTT CATTACAGCTC
ACGGTAACGA TGTCCTAGC ACCACAGTGC GAGCAGCAAA CCATACCGAA GTAAGTCGAG

7145 7150 7155 7160 7165 7170 7175 7180 7185 7190 7195 7200
*      *      *      *      *      *      *      *
CGGTTCCCAA CGATCAAGGC GAGTTACATG ATCCCCCATG TTGTGCAAAA AAGCGGTTAG
GCCAAGGGTT GCTAGTTCCG CTCAATGTAC TAGGGGTAC AACACGTTTT TTCGCCAATC

```

FIG. 29J

RECIPIED SHEET (RULE 91)

48/56

7205	7210	7215	7220	7225	7230	7235	7240	7245	7250	7255	7260
CTCCTTCGGT	CCTCCGATCG	TTGTCAGAAG	TAAGTTGGCC	GCAGTGTAT	CATCATGGT	GAGGAAGCCA	GGAGGCTAGC	AACAGTCTTC	ATTCAACCGG	CGTCACAATA	GTGAGTACCA
7265	7270	7275	7280	7285	7290	7295	7300	7305	7310	7315	7320
TATGGCAGCA	CTGCATAATT	CTCTTACTGT	CATGCCATCC	GTAAGATGCT	TTTCTGTGAC	ATACCGTCGT	GACGTATTAA	GAGAATGACA	GTACGGTAGG	CATTCTACGA	AAAGACACTG
7325	7330	7335	7340	7345	7350	7355	7360	7365	7370	7375	7380
TGGTGAGTAC	TCAACCAAGT	CATTCTGAGA	ATAGTGTATG	CGGCGACCGA	GTTGCTCTTG	ACCACTCATG	AGTTGGTTCA	GTAAGACTCT	TATCACATAC	GCCGCTGGCT	CAACGAGAAC
7385	7390	7395	7400	7405	7410	7415	7420	7425	7430	7435	7440
CCCGGCGTCA	ACACGGGATA	ATACCGCGCC	ACATAGCAGA	ACTTTAAAG	TGCTCATCAT	GGGCGCAGT	TGTGCCCTAT	TATGGCGCGG	TGTATCGTCT	TGAAATTTTC	ACGAGTAGTA
7445	7450	7455	7460	7465	7470	7475	7480	7485	7490	7495	7500
TGGAAAACGT	TCTTCGGGGC	GAAACTCTC	AAGGATCTTA	CCGCTGTTGA	GATCCAGTTC	ACCTTTTGCA	AGAAGCCCCG	CTTTTGAGAG	TTCCTAGAAT	GGCGACAACT	CTAGGTCAAG
7505	7510	7515	7520	7525	7530	7535	7540	7545	7550	7555	7560
GATGTAACCC	ACTCGTGCAC	CCAACCTGATC	TTCAGCATCT	TTTACTTTCA	CCAGCGTTTC	CTACATTGGG	TGAGCACGTG	GGTGACTAG	AAGTCGTAGA	AAATGAAAGT	GGTCGCAAG
7565	7570	7575	7580	7585	7590	7595	7600	7605	7610	7615	7620
TGGGTGAGCA	AAAACAGGAA	GGCAAAATGC	CGCAAAAAAG	GGAATAAGGG	CGACACGGAA	ACCCACTCGT	TTTTGTCTT	CCGTTTTACG	GCGTTTTTTC	CCTTATTCCC	GCTGTGCCTT
7625	7630	7635	7640	7645	7650	7655	7660	7665	7670	7675	7680
ATGTTGAATA	CTCATACTCT	TCCTTTTTCA	ATATTATTGA	AGCATTATC	AGGGTTATTG	TACAACTTAT	GAGTATGAGA	AGGAAAAAGT	TATAATAACT	TCGTAAATAG	TCCCAATAAC
7685	7690	7695	7700	7705	7710	7715	7720	7725	7730	7735	7740
TCTCATGAGC	GGATACATAT	TTGAATGTAT	TTAGAAAAAT	AAACAAATAG	GGGTCCCGCG	AGAGTACTCG	CCTATGTATA	AACTTACATA	AATCTTTTCA	TTTGTTTATC	CCCAAGGCGC
7745	7750	7755	7760	7765	7770	7775	7780	7785	7790	7795	7800
CACATTTCCC	CGAAAAGTGC	CACCTGACGT	CTAAGAAACC	ATTATTATCA	TGACATTAAAC	GTGTAAAGGG	GCTTTTCACG	GTGGACTGCA	GATTCTTTGG	TAATAATAGT	ACTGTAATTG
7805	7810	7815	7820	7825	7830	7835	7840	7845	7850	7855	7860
CTATAAAAT	AGGCGTATCA	CGAGGCCCTG	ATGGCTCTTT	GCGGCACCCA	TCGTTCTGTAA	GATATTTTCA	TCCGCATAGT	GCTCCGGGAC	TACCGAGAAA	CGCCGTGGGT	AGCAAGCATT
7865	7870	7875	7880	7885	7890	7895	7900	7905	7910	7915	7920
TGTTCCGTGG	CACCGAGGAC	AACCCTCAAG	AGAAAATGTA	ATCACACTGG	CTCACCTTGG	ACAAGGCACC	GTGGCTCCTG	TTGGGAGTTC	TCTTTTACAT	TAGTGTGACC	GAGTGGAAAGC

FIG. 29K

RECTIFIED SHEET (RULE 91)

49/56

7925	7930	7935	7940	7945	7950	7955	7960	7965	7970	7975	7980
GGTGGGCCTT	TCTGCGTTTA	TAAGGAGACA	CTTTATGTTT	AAGAAGGTTG	GTAATTCCT						
CCACCCGAA	AGACGCAAAT	ATTCCTCTGT	GAAATACAAA	TTCTTCCAAC	CATTTAAGGA						
7985	7990	7995	8000	8005	8010	8015	8020	8025	8030	8035	8040
TGCGGCTTTG	GCAGCCAAGC	TAGATCCGGC	TGTGGAATGT	GTGTCAAGTTA	GGGTGTGGAA						
ACGCCGAAAC	CGTCGGTTCG	ATCTAGGCCG	ACACCTTACA	CACAGTCAAT	CCCACACCTT						
8045	8050	8055	8060	8065	8070	8075	8080	8085	8090	8095	8100
AGTCCCCAGG	CTCCCCAGCA	GGCAGAAGTA	TGCAAAGCAT	GCATCTCAAT	TAGTCAGCAA						
TCAGGGGTCC	GAGGGGTTCG	CCGTCTTCAT	ACGTTTCGTA	CGTAGAGTTA	ATCAGTCGTT						
8105	8110	8115	8120	8125	8130	8135	8140	8145	8150	8155	8160
CCAGGCTCCC	CAGCAGGCAG	AAGTATGCAA	AGCATGCATC	TCAATTAGTC	AGCAACCATA						
GGTCCGAGGG	GTCGTCCGTC	TTCATACGTT	TCGTACGTAG	AGTTAATCAG	TCGTTGGTAT						
8165	8170	8175	8180	8185	8190	8195	8200	8205	8210	8215	8220
GTCCCGCCCC	TAATCCCGCC	CATCCCGCCC	CTAACTCCGC	CCAGTTCGCG	CCATTCTCCG						
CAGGGCGGGG	ATTGAGGCGG	GTAGGGCGGG	GATTGAGGCG	GGTCAAGGCG	GGTAAGAGCG						
8225	8230	8235	8240	8245	8250	8255	8260	8265	8270	8275	8280
CCCCATGGCT	GACTAATTTT	TTTTATTAT	GCAGAGGCCG	AGGCCGCCTC	GGCCTCTGAG						
GGGGTACCGA	CTGATTAATA	AAAATAAATA	CGTCTCCGGC	TCCGGCGGAG	CCGGAGACTC						
8285	8290	8295	8300	8305	8310	8315	8320	8325	8330	8335	8340
CTATTCCAGA	AGTAGTGAGG	AGGCTTTTTT	GGAGGCCTAG	GCTTTTGCAA	AAACTAGCTT						
GATAAGGTCT	TCATCACTCC	TCCGAAAAAA	CCTCCGGATC	CGAAAACGTT	TTTGATCGAA						
8345	8350	8355	8360	8365	8370	8375	8380	8385	8390	8395	8400
GGGGCCACCG	CTCAGAGCAC	CTTCCACCAT	GGCCACCTCA	GCAAGTTCCC	ACTTGAACAA						
CCCCGGTGGC	GAGTCTCGTG	GAAGGTGGTA	CCGGTGGAGT	CGTTCAAGGG	TGAACCTGTT						
8405	8410	8415	8420	8425	8430	8435	8440	8445	8450	8455	8460
AAACATCAAG	CAAATGTACT	TGTGCCTGCC	CCAGGGTGAG	AAAGTCCAAG	CCATGTATAT						
TTTGTAGTTC	GTTTACATGA	ACACGGACGG	GGTCCCACTC	TTTCAGGTTT	GGTACATATA						
8465	8470	8475	8480	8485	8490	8495	8500	8505	8510	8515	8520
CTGGGTTGAT	GGTACTGGAG	AAGGACTCCG	CTGCAAAACC	CGCACCTGG	ACTGTGAGCC						
GACCCAATA	CCATGACCTC	TTCCTGACGC	GACGTTTGG	GCGTGGGACC	TGACACTCCG						
8525	8530	8535	8540	8545	8550	8555	8560	8565	8570	8575	8580
CAAGTGTGTA	GAAGAGTTAC	CTGAGTGGAA	TTTTGATGGC	TCTAGTACCT	TTCAGTCTTA						
GTTACACAT	CTTCTCAATG	GACTCACCTT	AAAACACTCCG	AGATCATGGA	AAGTCAGAT						
8585	8600	8595	9600	8605	8610	8615	8620	8625	8630	8635	8640
GGGCTCCAAC	AGTGACATGT	ATCTCAGCCC	TGTTGCCATG	TTTCGGGACC	CCTTCCG						
CCCAGGTTG	TCACTGTACA	TAGAGTCGGG	ACAACGGTAC	AAAGCCCTGG	GGAAGGCT						

FIG. 29L

REPTILES SHEET (RULE 91)

50/56

8645	8650	8655	8660	8665	8670	8675	8680	8685	8690	8695	8700
AGATCCCAAC	AAGCTGGTGT	TCTGTGAAGT	TTTCAAGTAC	AACCGGAAGC	CTGCAGAGAC	TCTAGGGTTG	TTCGACCACA	AGACACTTCA	AAAGTTCATG	TTGGCCTTCG	GACGTCTCTG
8705	8710	8715	8720	8725	8730	8735	8740	8745	8750	8755	8760
CAATTTAAGG	CACTCGTGTA	AACGGATAAT	GGACATGGTG	AGCAACCAGC	ACCCCTGGTT	GTAAATTC	GTGAGCACAT	TTGCCTATT	CCTGTACCAC	TCGTTGGTCG	TGGGGACCAA
8765	8770	8775	8780	8785	8790	8795	8800	8805	8810	8815	8820
TGGAATGGAA	CAGGAGTATA	CTCTGATGGG	AACAGATGGG	CACCCTTTTG	GTTGGCCTTC	ACCTTACCTT	GTCCTCATAT	GAGACTACCC	TTGTCTACCC	GTGGGAAAAC	CAACCGGAAG
8825	8830	8835	8840	8845	8850	8855	8860	8865	8870	8875	8880
CAATGGCTTT	CCTGGGCCCC	AAGGTCCGTA	TTACTGTGGT	GTGGGCGCAG	ACAAAGCCTA	GTTACCGAAA	GGACCCGGGG	TTCCAGGCAT	AATGACACCA	CACCCGCGTC	TGTTTCGGAT
8885	8890	8895	8900	8905	8910	8915	8920	8925	8930	8935	8940
TGGCAGGGAT	ATCGTGGAGG	CTCACTACCG	CGCCTGCTTG	TATGCTGGGG	TCAAGATTAC	ACCGTCCCTA	TAGCACCTCC	GAGTGATGGC	GCGGACGAAC	ATACGACCCC	AGTTCTAATG
8945	8950	8955	8960	8965	8970	8975	8980	8985	8990	8995	9000
AGGAACAAAT	GCTGAGGTCA	TGCCTGCCCA	GTGGGAACTC	CAAATAGGAC	CCTGTGAAGG	TCCTTGTTTA	CGACTCCAGT	ACGGACGGGT	CACCCTTGAG	GTTTATCCTG	GGACACTTCC
9005	9010	9015	9020	9025	9030	9035	9040	9045	9050	9055	9060
AATCCGCATG	GGAGATCATC	TCTGGGTGGC	CCGTTTCATC	TTNCATCGAG	TATGTGAAGA	TTAGGCGTAC	CCTCTAGTAG	AGACCCACCG	GGCAAACCTAG	AANGTAGCTC	ATACACTTCT
9065	9070	9075	9080	9085	9090	9095	9100	9105	9110	9115	9120
CTTTGGGGTA	ATAGCAACCT	TTGACCCCAA	GCCCATTCTC	GGGAACTGGA	ATGGTGCCAGG	GAAACCCCAT	TATCGTTGGA	AACTGGGGTT	CGGGTAAGGA	CCCTTGACCT	TACCACGTCC
9125	9130	9135	9140	9145	9150	9155	9160	9165	9170	9175	9180
CTGCCATACC	AACTTTAGCA	CCAAGGCCAT	GCGGGAGGAG	AATGGTCTGA	AGCACATCGA	GACGGTATGG	TTGAAATCGT	GGTTCCGGTA	CGCCCTCCTC	TTACCAGACT	TCGTGTAGCT
9185	9190	9195	9200	9205	9210	9215	9220	9225	9230	9235	9240
GGAGGCCATC	GAGAACTAA	GCAAGCGGCA	CCGGTACCAC	ATTCGAGCCT	ACGATCCCAA	CCTCCGGTAG	CTCTTTGATT	CGTTCCGGT	GGCCATGGTG	TAAGCTCGGA	TGCTAGGGTT
9245	9250	9255	9260	9265	9270	9275	9280	9285	9290	9295	9300
GGGGGGCCTG	GACAATGCCC	GTGGTCTGAC	TGGGTTCCAC	GAAACGTCCA	ACATCAACGA	CCCCCGGAC	CTGTTACGGG	CACCAGACTG	ACCCAAGGTG	CTTTGCAGGT	TGTAGTTGCT
9305	9310	9315	9320	9325	9330	9335	9340	9345	9350	9355	9360
CTTTTCTGCT	GGTGTCCGCA	ATCGCAGTGC	CAGCATCCGC	ATTCCCCGGA	CTGTCCGGCA	GAAAAGACGA	CCACAGCGGT	TAGCGTCACG	GTCGTAGGCG	TAAGGGGCCT	GACAGCCGGT

FIG. 29M

RECTIFIED SHEET (RULE 91)

51/56

9365	9370	9375	9380	9385	9390	9395	9400	9405	9410	9415	9420
GGAGAAGAAA	GGTTACTTTG	AAGACCGCGG	CCCCTCTGCC	AATTGTGACC	CCTTTGCAGT	CCTCTTCTTT	CCAATGAAAC	TTCTGGCGCC	GGGGAGACGG	TTAACACTGG	GGAAACGTCA
9425	9430	9435	9440	9445	9450	9455	9460	9465	9470	9475	9480
GACAGAAGCC	ATCGTCCGCA	CATGCCTTCT	CAATGAGACT	GGCCACGAGC	CCTTCCAATA	CTGTCTTCGG	TAGCAGGCGT	GTACGGAAGA	GTTACTCTGA	CCGGTGCTCG	GGAAGGTTAT
9485	9490	9495	9500	9505	9510	9515	9520	9525	9530	9535	9540
CAAAAACATA	TTAGACTTTG	AGTGATCTTG	AGCCTTTCCT	AGTTCATCCC	ACCCCGCCCC	GTTTTTGATT	AATCTGAAAC	TCACTAGAAC	TCGGAAAGGA	TCAAGTAGGG	TGGGGCGGGG
9545	9550	9555	9560	9565	9570	9575	9580	9585	9590	9595	9600
AGAGAGATCT	TTGTGAAGGA	ACCTTACTTC	TGTGGTGTGA	CATAATTGGA	CAAACCTACCT	TCTCTCTAGA	AACACTTCCT	TGGAATGAAG	ACACCACACT	GTATTAACCT	GTTTGATGGA
9605	9610	9615	9620	9625	9630	9635	9640	9645	9650	9655	9660
ACAGAGATTT	AAAGCTCTAA	GGTAAATATA	AAATTTTAA	GTGTATAATG	TGTTAAACTA	TGTCTCTAAA	TTTCGAGATT	CCATTTATAT	TTTAAAAATT	CACATATTAC	ACAATTTGAT
9665	9670	9675	9680	9685	9690	9695	9700	9705	9710	9715	9720
CTGATTCTAA	TTGTTTGTGT	ATTTTAGATT	CCAACCTATG	GAAGTATGTA	ATGGGAGCAG	GACTAAGATT	AACAAACACA	TAAAATCTAA	GGTTGGATAC	CTTGACTACT	TACCCTCGTC
9725	9730	9735	9740	9745	9750	9755	9760	9765	9770	9775	9780
TGGTGAATG	CCTTTAATGA	GGAAAACCTG	TTTTGCTCAG	AAGAAATGCC	ATCTAGTGAT	ACCACCTTAC	GGAAATTACT	CCTTTTGGAC	AAAACGAGTC	TTCTTTACGG	TAGATCACTA
9785	9790	9795	9800	9805	9810	9815	9820	9825	9830	9835	9840
GATGAGGCTA	CTGCTGACTC	TCAACATTCT	ACTCCTCCAA	AAAAGAAGAG	AAAGGTAGAA	CTACTCCGAT	GACGACTGAG	AGTTGTAAGA	TGAGGAGGTT	TTTTCTTCTC	TTTCCATCTT
9845	9850	9855	9860	9865	9870	9875	9880	9885	9890	9895	9900
GACCCCAAGG	ACTTTCCTTC	AGAATTGCTA	AGTTTTTTGA	GTCATGCTGT	GTTTAGTAAT	CTGGGGTTCC	TGAAAGGAAG	TCTTAACGAT	TCAAAAAACT	CAGTACGACA	CAAATCATTAA
9905	9910	9915	9920	9925	9930	9935	9940	9945	9950	9955	9960
AGAACTCTTG	CTTGCTTTGC	TATTTACACC	ACAAAGGAAA	AAGCTGCACT	GCTATACAAG	TCTTGAGAAC	GAACGAAACG	ATAAATGTGG	TGTTTCCTTT	TTGACGTGA	CGATATGTTT
9965	9970	9975	9980	9985	9990	9996	10000	10005	10010	10015	10020
AAAATTATGG	AAAAATATTC	TGTAACCTTT	ATAAGTAGGC	ATAACAGTTA	TAATCATAAC	TTTTAATACC	TTTTTATAAG	ACATTGAAAA	TATTCATCCG	TATTGTCAAT	ATTAGTATTG
10025	10030	10035	10040	10045	10050	10055	10060	10065	10070	10075	10080
ATACTGTTTT	TTCTTACTCC	ACACAGGCAT	AGAGTGTCTG	CTATTAATAA	CTATGCTCAA	TATGACAAAA	AAGAATGAGC	TGTGTCCGTA	TCTCACAGAC	GATAATTATT	GATACGAGTT

FIG. 29N

RECTIFIED SHEET (P. 1)

52/56

1008510090	1009510100	1010510110	1011510120	1012510130	1013510140
AAATTGTGTA	CCTTTAGCTT	TTTAATTTGT	AAAGGGGTTA	ATAAGGAATA	TTTGATGTAT
TTTAACACAT	GGAAATCGAA	AAATTAAACA	TTTCCCAAT	TATTCCTTAT	AAACTACATA
1014510150	1015510160	1016510170	1017510180	1018510190	1019510200
AGTGCCTTGA	CTAGAGATCA	TAATCAGCCA	TACCACATT	GTAGAGGTTT	TACTTGCTTT
TCACGGAAC	GATCTCTAGT	ATTAGTCGGT	ATGGTGTA	CATCTCCAAA	ATGAACGAAA
1020510210	1021510220	1022510230	1023510240	1024510250	1025510260
AAAAAACCTC	CCACACCTCC	CCCTGAACCT	GAAACATAAA	ATGAATGCAA	TTGTTGTTGT
TTTTTTGGAG	GGTGTGGAGG	GGGACTTGGA	CTTTGTATTT	TACTTACGTT	AACAACAACA
1026510270	1027510280	1028510290	1029510300	1030510310	1031510320
TAACTTGTTT	ATTGCAGCTT	ATAATGGTTA	CAAATAAAGC	AATAGCATCA	CAAATTTTAC
ATTGAACAAA	TAACGTCGAA	TATTACCAAT	GTTTATTTTCG	TTATCGTAGT	GTTTAAAGTG
1032510330	1033510340	1034510350	1035510360	1036510370	1037510380
AAATAAAGCA	TTTTTTTTCAC	TGCATTCTAG	TTGTGGTTTG	TCCAAACTCA	TCAATGTATC
TTTATTTCTG	AAAAAAAGTG	ACGTAAGATC	AACACCAAAC	AGGTTTGAGT	AGTTACATAG
1038510390	1039510400	1040510410	1041510420	1042510430	1043510440
TTATCATGTC	TGGATCTCTA	GCTTCGTGTC	AAGGACGGTG	ACTGCAGTGA	ATAATAAAAT
AATAGTACAG	ACCTAGAGAT	CGAAGCACAG	TTCCTGCCAC	TGACGTCACT	TATTATTTTIA
1044510450	1045510460	1046510470	1047510480	1048510490	1049510500
GTGTGTTTGT	CCGAAATACG	CGTTTGTGGA	TTTCTGTGCG	CGACTAAATT	CATGTCGCGC
CACACAAACA	GGCTTTATGC	GCAAACTCT	AAAGACAGCG	GCTGATTAA	GTACAGCGCG
1050510510	1051510520	1052510530	1053510540	1054510550	1055510560
GATAGTGGTG	TTTATCGCCG	ATAGAGATGG	CGATATTGGA	AAAATCGATA	TTTGAAAATA
CTATCACCAC	AAATAGCGGC	TATCTCTACC	GCTATAACCT	TTTATAGCTAT	AAACTTTTAT
1056510570	1057510580	1058510590	1059510600	1060510610	1061510620
TGGCATATTG	AAAATGTCGC	CGATGTGAGT	TTCTGTGTAA	CTGATATCGC	CATTTTTTCCA
ACCGTATAAC	TTTTACAGCG	GCTACACTCA	AAGACACATT	GACTATAGCG	GTAATAAGGT
1062510630	1063510640	1064510650	1065510660	1066510670	1067510680
AAAGTGATTT	TTGGGCATAC	GCGATATCTG	GCGATAGCGC	TTATATCGTT	TACGGGGGAT
TTTCACTAAA	AACCCGTATG	CGCTATAGAC	CGCTATCGCG	AATATAGCAA	ATGCCCCCTA
1068510690	1069510700	1070510710	1071510720	1072510730	1073510740
GGCGATAGAC	GACTTTGGTG	ACTTGGGCGA	TTCTGTGTGT	CGCAAATATC	GCAGTTTTCGA
CCGCTATCTG	CTGAAACAC	TGAACCCGCT	AAGACACACA	GCGTTTATAG	CGTCAAAGCT
1074510750	1075510760	1076510770	1077510780	1078510790	1079510800
TATAGGTGAC	AGACGATATG	AGGCTATATC	GCCGATAGAG	GCGACATCAA	GCTGGCACAT
ATATCCACTG	TCTGCTATAC	TCCGATATAG	CGGCTATCTC	CGCTGTAGTT	CGACCGTGTA

FIG. 290

RECTIFIED SHEET (RULE 91)

53/56

1080510810 1081510820 1082510830 1083510840 1084510850 1085510860
* * * * *
GGCCAATGCA TATCGATCTA TACATTGAAT CAATATTGGC CATTAGCCAT ATTATTCATT
CCGGTTACGT ATAGCTAGAT ATGTAACCTA GTTATAACCG GTAATCGGTA TAATAAGTAA

1086510870 1087510880 1088510890 1089510900 1090510910 1091510920
* * * * *
GGTTATATAG CATAAATCAA TATTGGCTAT TGGCCATTGC ATACGTTGTA TCCATATCAT
CCAATATATC GTATTTAGTT ATAACCGATA ACCGGTAACG TATGCAACAT AGGTATAGTA

1092510930 1093510940 1094510950 1095510960 1096510970 1097510980
* * * * *
AATATGTACA TTTATATTGG CTCATGTCCA ACATTACCGC CATGTTGACA TTGATTATTG
TTATACATGT AAATATAACC GAGTACAGGT TGTAATGGCG GTACAACTGT AACTAATAAC

1098510990 1099511000 1100511010 1101511020 1102511030 1103511040
* * * * *
ACTAGTTATT AATAGTAATC AATTACGGGG TCATTAGTTC ATAGCCCATA TATGGAGTTC
TGATCAATAA TTATCATTAG TTAATGCCCC AGTAATCAAG TATCGGGTAT ATACCTCAAG

1104511050 1105511060 1106511070 1107511080 1108511090 1109511100
* * * * *
CGCGTTACAT AACTTACGGT AAATGGCCCC CCTGGCTGAC CGCCCAACGA CCCCCGCCA
GCGCAATGTA TTGAATGCCA TTTACCGGGC GGACCGACTG GCGGGTTGCT GGGGGCGGGT

1110511110 1111511120 1112511130 1113511140 1114511150 1115511160
* * * * *
TTGACGTCAA TAATGACGTA TGTTCACATA GTAACGCCAA TAGGGACTTT CCATTGACGT
AACTGCAGTT ATTACTGCAT ACAAGGGTAT CATTGCGGTT ATCCCTGAAA GGTAAGTGA

1116511170 1117511180 1118511190 1119511200 1120511210 1121511220
* * * * *
CAATGGGTGG AGTATTTACG GTAAACTGCC CACTTGCCAG TACATCAAGT GTATCATATG
GTTACCCACC TCATAAATGC CATTTGACGG GTGAACCGTC ATGTAGTTCA CATAGTATAC

1122511230 1123511240 1124511250 1125511260 1126511270 1127511280
* * * * *
CCAAGTACGC CCCCTATTGA CGTCAATGAC GGTAAATGGC CCGCCTGGCA TTATGCCAG
GGTTCATGCG GGGGATAACT GCAGTTACTG CCATTTACCG GGCGGACCGT AATACGGGTC

1128511290 1129511300 1130511310 1131511320 1132511330 1133511340
* * * * *
TACATGACCT TATGGGACTT TCCTACTTGG CAGTACATCT ACGTATTAGT CATCGCTATT
ATGTACTGGA ATACCCTGAA AGGATGAACC GTCATGTAGA TGCATAATCA GTAGCGATAA

1134511350 1135511360 1136511370 1137511380 1138511390 1139511400
* * * * *
ACCATGGTGA TGCGGTTTGG GCAGTACATC AATGGGCGTG GATAGCGGTT TGAATCACGG
TGGTACCACT ACGCCAAAAC CGTCATGTAG TTACCCGCAC CTATCGCCAA ACTGAGTGCC

1140511410 1141511420 1142511430 1143511440 1144511450 1145511460
* * * * *
GGATTTCCAA GTCTCCACCC CATTGACGTC AATGGGAGTT TGTTTTGGCA CCAAAATCAA
CCTAAAGGTT CAGAGGTGGG GTAAGTGCAG TTACCCTCAA ACAAACCGT GGTTTTAGTT

1146511470 1147511480 1148511490 1149511500 1150511510 1151511520
* * * * *
CGGGACTTTC CAAAATGTCT TAACAACCTC GCCCCATTGA CGCAAATGGG CGGTAGGCGT
GCCCTGAAAG GTTTTACAGC ATTGTTGAGG CGGGGTAACG GCGTTTACCC GCCATCCGCA

FIG. 29P

RECTIFIED SHEET (RULE 90)

54/56

1152511530	1153511540	1154511550	1155511560	1156511570	1157511580
GTACGGTGGG	AGGTCTATAT	AAGCAGAGCT	CGTTTAGTGA	ACCGTCAGAT	CGCCTGGAGA
CATGCCACCC	TCCAGATATA	TTCGTCTCGA	GCAAATCACT	TGGCAGTCTA	GCGGACCTCT
1158511590	1159511600	1160511610	1161511620	1162511630	1163511640
CGCCATCCAC	GCTGTTTTGA	CCTCCATAGA	AGACACCGGG	ACCGATCCAG	CCTCCGCGGC
GCGGTAGGTG	CGACAAAAC	GGAGGTATCT	TCTGTGGCCC	TGGCTAGGTC	GGAGGCGCCC
1164511650	1165511660	1166511670	1167511680	1168511690	1169511700
CGGGAACGGT	GCATTGGAAC	GCGGATTCCC	CGTGCCAAGA	GTGACGTAAG	TACCGCCTAT
GCCCTTGCCA	CGTAACCTTG	CGCCTAAGGG	GCACGGTTCT	CACTGCATTG	ATGGCGGATA
1170511710	1171511720	1172511730	1173511740	1174511750	1175511760
AGAGTCTATA	GGCCCACCCC	CTTGGCTTCT	TATGCATGCT	ATACTGTTTT	TGGCTTGGGG
TCTCAGATAT	CCGGGTGGGG	GAACCGAAGA	ATACGTACGA	TATGACAAA	ACCGAACCCC
1176511770	1177511780	1178511790	1179511800	1180511810	1181511820
TCTATACACC	CCCGCTTCCT	CATGTTATAG	GTGATGGTAT	AGCTTAGCCT	ATAGGTGTGG
AGATATGTGG	GGGCGAAGGA	GTACAATATC	CACTACCATA	TGAATCGGA	TATCCACACC
1182511830	1183511840	1184511850	1185511860	1186511870	1187511880
GTTATTGACC	ATTATTGACC	ACTCCCCTAT	TGGTGACGAT	ACTTTCATT	ACTAATCCAT
CAATAACTGG	TAATAACTGG	TGAGGGGATA	ACCACTGCTA	TGAAAGGTAA	TGATTAGGTA
1188511890	1189511900	1190511910	1191511920	1192511930	1193511940
AACATGGCTC	TTTGCCACAA	CTCTCTTTAT	TGGCTATATG	CCAATACACT	GTCCTTCAGA
TTGTACCGAG	AAACGGTGTT	GAGAGAAATA	ACCGATATAC	GGTTATGTGA	CAGGAAGTCT
1194511950	1195511960	1196511970	1197511980	1198511990	1199512000
GACTGACACG	GACTCTGTAT	TTTTACAGGA	TGGGGTCTCA	TTTATTATTT	ACAAATTCAC
CTGACTGTGC	CTGAGACATA	AAAATGTCTT	ACCCGAGAGT	AAATAATAAA	TGTTTAAAGT
1200512010	1201512020	1202512030	1203512040	1204512050	1205512060
ATATACAACA	CCACCGTCCC	CAGTGCCCGC	AGTTTTTATT	AAACATAACG	TGGGATCTCC
TATATGTTGT	GGTGGCAGGG	GTCACGGGCG	TCAAAAATAA	TTTGTATTGC	ACCCTAGAGG
1206512070	1207512080	1208512090	1209512100	1210512110	1211512120
ACGCGAATCT	CGGGTACGTG	TTCCGGACAT	GGGCTCTTCT	CCGGTAGCGG	CGGAGCTTCT
TGCGCTTAGA	GCCCATGCAC	AAGGCCTGTA	CCCAGAGAAG	GGCCATCGCC	GCCTCGAAGA
1212512130	1213512140	1214512150	1215512160	1216512170	1217512180
ACATCCGAGC	CCTGCTCCCA	TGCCTCCAGC	GACTCATGGT	CGCTCGGCAG	CTCCTTCTCT
TGTAGGCTCG	GGACGAGGGT	ACGGAGGTCT	CTGAGTACCA	GCGAGCCGTC	GAGGAAATTA
1218512190	1219512200	1220512210	1221512220	1222512230	1223512240
CTAACAGTGG	AGGCCAGACT	TAGGCACAGC	ACGATGCCCA	CCACCACCAG	TGTGCCCTTA
GATTGTCACC	TCCGGTCTGA	ATCCGTGTCT	TGCTACGGGT	GGTGGTGGTC	ACACGGGCTT

FIG. 29Q

RESERVED SHEET PAGE 90

55/56

1224512250 1225512260 1226512270 1227512280 1228512290 1229512300
* * * * *
AAGGCCGTGG CGGTAGGGTA TGTGTCTGAA AATGAGCTCG GGGAGCGGGC TTGCACCGCT
TTCCGGCACC GCCATCCCAT ACACAGACTT TACTCGAGC CCCTCGCCCG AACGTGGCGA
1230512310 1231512320 1232512330 1233512340 1234512350 1235512360
* * * * *
GACGCATTG GAAGACTTAA GGCAGCGGCA GAAGAAGATG CAGGCAGCTG AGTTGTTGTG
CTGCGTAAAC CTTCTGAATT CCGTCGCCGT CTTCTTCTAC GTCCGTCGAC TCAACAACAC
1236512370 1237512380 1238512390 1239512400 1240412410 1241512420
* * * * *
TTCTGATAAG AGTCAGAGGT AACTCCCGTT GCGGTGCTGT TAACGGTGGA GGGCAGTGTA
AAGACTATTC TCAGTCTCCA TTGAGGGCAA CGCCACGACA ATTGCCACCT CCCGTCACAT
1242512430 1243512440 1244512450 1245512460 1246512470 1247512480
* * * * *
GTCTGAGCAG TACTCGTTGC TGCCGCGCGC GCCACCAGAC ATAATAGCTG ACAGACTAAC
CAGACTCGTC ATGAGCAACG ACGGCGCGCG CGGTGGTCTG TATTATCGAC TGTCTGATTG
1248512490 1249512500 1250512510 1251512520 1252512530 1253512540
* * * * *
AGACTGTTCC TTTCCATGGG TCTTTTCTGC AGTCACCGTC CTTGACACGA AGCTTACCAT
TCTGACAAGG AAAGGTACCC AGAAAAGACG TCAGTGGCAG GAACTGTGCT TCGAATGGTA
1254512550 1255512560 1256512570 1257512580 1258512590 1259512600
* * * * *
GGGTGTGCCC ACTCAGGTCC TGCGGTGCT GCTGCTGTGG CTTACAGATG CCAGATGTGA
CCCACACGGG TGAGTCCAGG ACCCCAACGA CGACGACACC GAATGTCTAC GGTCTACACT
1260512610 1261512620 1262512630 1263512640 1264512650 1265512660
* * * * *
GATCGTTCTC ACGCAGTCTC CAGGCACCTT GTCTCTGTCT CCAGGGGAAA GAGCCACCTT
CTAGCAAGAG TGCGTCAGAG GTCCGTGGGA CAGAGACAGA GGTCCCCTTT CTCGGTGGAA
1266512670 1267512680 1268512690 1269512700 1270512710 1271512720
* * * * *
CTCCTGTAGG TCCAGTCACA GCATTGCGAG CCGCCGCGTA GCCTGGTACC AGCACAAACC
GAGGACATCC AGGTCAGTGT CGTAAGCGTC GCGGCGGCAT CGGACCATGG TCGTGTGTTG
1272512730 1273512740 1274512750 1275512760 1276512770 1277512780
* * * * *
TGGCCAGGCT CCAAGGCTGG TCATACATGG TGTTCCTCAAT AGGGCCTCTG GCATCTCAGA
ACCGGTCCGA GGTTCGGACC AGTATGTACC ACAAAGGTTA TCCCGGAGAC CGTAGAGTCT
1278512790 1279512800 1280512810 1281512820 1282512830 1283512840
* * * * *
CAGGTTTCAG GGCAGTGGGT CTGGGACAGA CTTCACTCTC ACCATCACCA GAGTGGAGCC
GTCCAAGTCG CCGTCACCCA GACCCTGTCT GAAGTGAGAG TGGTAGTGGT CTCACCTCGG
1284512850 1285512860 1286512870 1287512880 1288512890 1289512900
* * * * *
TGAAGACTTT GCACTGTACT ACTGTCAGGT CTATGGTGCC TCCTCGTACA CTTTGGCCA
ACTTCTGAAA CGTGACATGA TGACAGTCCA GATACCACGG AGGAGCATGT GAAAACCGGT
1290512910 1291512920 1292512930 1293512940 1294512950 1295512960
* * * * *
GGGGACCAAA CTGGAGAGGA AACGAAGTGT GCCTGCACCA TCTGTCTTCA TCTTCCCGCC
CCCCTGGTTT GACCTCTCCT TTGCTTGACA CGGACGTGGT AGACAGAAGT AGAAGGGCGG

FIG. 29R

RECTIFIED G-SEQUENCE (30)

56/56

1296512970	1297512980	1298512990	1299513000	1300513010	1301513020
*	*	*	*	*	*
ATCTGATGAG	CAGTTGAAAT	CTGGGACTGC	CTCTGTTGTG	TGCCTGCTGA	ATAACTTCTA
TAGACTACTC	GTCAACTTTA	GACCCTGACG	GAGACAACAC	ACGGACGACT	TATTGAAGAT
1302513030	1303513040	1304513050	1305513060	1306513070	1307513080
*	*	*	*	*	*
TCCCAGAGAG	GCCAAAGTAC	AGTGGAAGGT	GGATAACGCC	CTCCAATCGG	GTAAC TCCCA
AGGGTCTCTC	CGGTTTCATG	TCACCTTCCA	CCTATTGCGG	GAGGTTAGCC	CATTGAGGGT
1308513090	1309513100	1310513110	1311513120	1312513130	1313513140
*	*	*	*	*	*
GGAGAGTGTG	ACAGAGCAGG	ACAGCAAGGA	CAGCACCTAC	AGCCTCAGCA	GCACCCTGAC
CCTCTCACAG	TGTCTCGTCC	TGTCGTTCCCT	GTCGTGGATG	TCGGAGTCGT	CGTGGGACTG
1314513150	1315513160	1316513170	1317513180	1318513190	1319513200
*	*	*	*	*	*
GCTGAGCAAA	GCAGACTACG	AGAAACACAA	AGTCTACGCC	TGCGAAGTCA	CCCATCAGGG
CGACTCGTTT	CGTCTGATGC	TCTTTGTGTT	TCAGATGCGG	ACGCTTCAGT	GGGTAGTCCC
1320513210	1321513220	1322513230	1323513240	1324513250	13255
*	*	*	*	*	
CCTGAGATCG	CCCGTCACAA	AGAGCTTCAA	CAGGGGAGAG	TGTTAATTCT	AGAGAA
GGACTCTAGC	GGGCAGTGTT	TCTCGAAGTT	GTCCCTCTC	ACAATTAAGA	TCTCTT

FIG. 29S

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/08743

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : A61K 39/42; C07K 16/10; G01N 33/577
US CL : 424/142.1, 148.1; 435/7.93; 530/388.15, 388.35.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
SEQUENCE SEARCH (GeneSeq, PIR, Swiss-Prot), APS, DIALOG.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, USA, Vol. 89, issued October 1992, Barbas et al., "Recombinant human Fab fragments neutralize human type 1 immunodeficiency virus in vitro," pages 9339-9343, see entire document.	1-7, 9-11, 15, 29 ----- 8, 30
X -- Y	JOURNAL OF MOLECULAR BIOLOGY, Vol. 230, issued 1993, Barbas et al., "Molecular profile of an antibody response to HIV-1 as probed by combinatorial libraries," pages 812-823, see entire document.	1-7, 9-11, 15, 29 ----- 8, 30



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

28 SEPTEMBER 1995

Date of mailing of the international search report

12 OCT 1995

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231Authorized officer
ROBERT D. BUDENS

Facsimile No. (703) 305-3230

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/08743

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, USA, Vol. 91, issued April 1994, Barbas et al., "In vitro evolution of a neutralizing human antibody to human immunodeficiency virus type 1 to enhance affinity and broaden strain cross-reactivity," pages 3809-3813, see entire document.	1-7, 9-11 ----- 8, 15, 29-30
X,P ----- Y,P	JOURNAL OF VIROLOGY, Vol. 68, No. 8, issued August 1994, Roben et al., "Recognition properties of a panel of human recombinant Fab Fragments to the CD4 binding site of gp120 that show differing abilities to neutralize human immunodeficiency virus type 1," pages 4821-4828, see entire document.	1-7, 9-11, 29 ----- 8, 15, 30
X,P ----- Y,P	SCIENCE, Vol. 266, issued 11 November 1994, Burton et al., "Efficient Neutralization of primary isolates of HIV-1 by a recombinant human monoclonal antibody," pages 1024-1027, see entire document.	1-11, 29 ----- 15, 30
X,P ----- Y,P	JOURNAL OF VIROLOGY, Vol. 69, No. 1, issued January 1995, Moore et al., "Primary isolates of human immunodeficiency virus type 1 are relatively resistant to neutralization by monoclonal antibodies to gp120, and their neutralization is not predicted by studies with monomeric gp120," pages 101-109, see entire document.	1-11, 29 ----- 15, 30
Y	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, USA, Vol. 88, issued November 1991, Burton et al., "A large array of human monoclonal antibodies to type 1 human immunodeficiency virus from combinatorial libraries of asymptomatic seropositive individuals," pages 10134-10137, see entire document.	1-11, 15, 29-30
Y	HUMAN ANTIBODIES AND HYBRIDOMAS, Vol. 4, issued April 1993, Bender et al., "Recombinant human antibodies: linkage of an Fab fragment from a combinatorial library to an Fc fragment for expression in mammalian cell culture," pages 74-79, see entire document.	1-11, 15, 29-30

INTERNATIONAL SEARCH REPORT

Inte.national application No.
PCT/US95/08743

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-11, 15, 29-30

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/08743

B. FIELDS SEARCHED

Minimum documentation searched

Classification System: U.S.

424/134.1, 142.1, 148.1, 160.1, 188.1; 435/7.92, 7.93, 7.94, 7.95, 69.6, 69.7, 172.2, 172.3, 240.2, 240.27, 252.3, 252.33, 320.1; 530/387.3, 388.15, 388.35, 389.4.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-11, 15 and 29-30, drawn to anti-gp120 human monoclonal antibodies, a first method of use, pharmaceutical compositions and a kit.

Group II, claim(s) 12-14, drawn to nucleotides, vectors and host cells.

Group III, claim(s) 16-21, drawn to a second method of use, methods of diagnosis.

Group IV, claim(s) 22-28, drawn to a third method of use, methods of passive immunotherapy.

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the inventions of Groups I and II are directed to distinct products differing in their physical, chemical and immunological activities and are not so linked by a special technical feature within the meaning of PCT Rule 13.2 as to form a single general inventive concept.

The inventions listed as Groups I and (III-IV) do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the inventions are related as product and method of use and the product of Group I can be used for multiple purposes as evidenced by the inventions of Groups III-IV and are not so linked by a special technical feature within the meaning of PCT Rule 13.2 as to form a single general inventive concept.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be examined, the appropriate additional examination fees must be paid. The species are as follows:

Species A, claims 16-18, directed to methods of in vivo diagnosis.

Species B, claims 16 and 19-21, directed to methods of in vitro diagnosis.

The following claims are generic: claim 16.

The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: Species A and B are directed to distinct methods having different industrial applicabilities as well as utilizing different reagents and method steps and are not so linked by a special technical feature within the meaning of PCT Rule 13.2 as to form a single general inventive concept.